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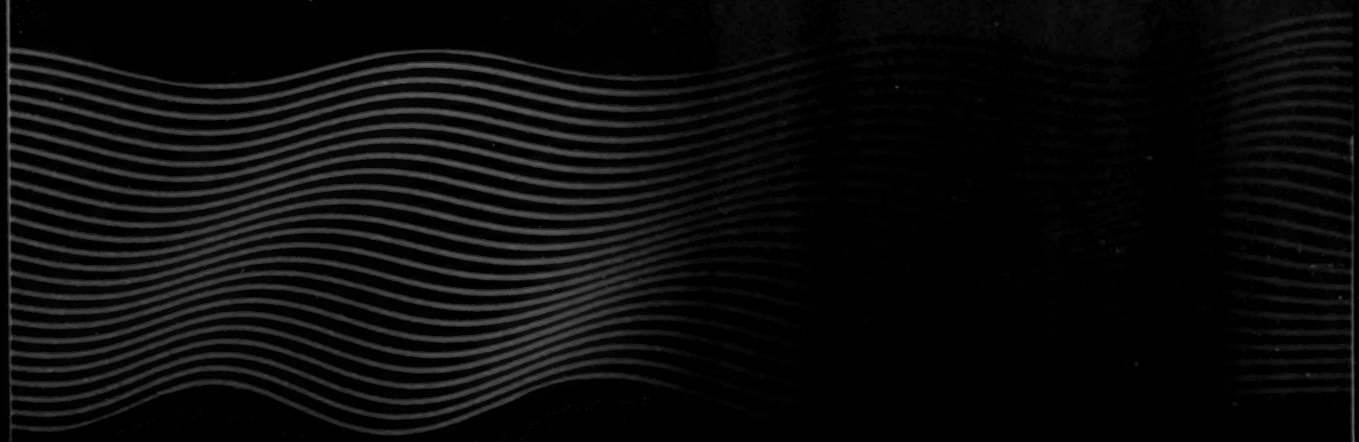
Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques



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The *Canadian Journal of Fisheries and Aquatic Sciences*, previously published as *Contributions to Canadian Biology* 1901–1925, *Contributions to Canadian Biology and Fisheries* 1926–1934, *Journal of the Biological Board of Canada* 1934–1937, and *Journal of the Fisheries Research Board of Canada* 1938–1979, is issued monthly to form annual volumes of 12 numbers plus supplements.

Editorial policy: The Journal publishes original research articles, perspectives (syntheses, critiques, re-evaluations), discussions (comment and reply), and rapid communications. Manuscripts may concern cells, organisms, populations, communities, ecosystems, or processes that affect aquatic systems. They may cover a range of disciplines including biology and ecology of marine and freshwater organisms, limnology, oceanography, physiology, toxicology, genetics, economics, disease, and management. Preference will be given to manuscripts that emphasize understanding of observed phenomena and interpretation of experimental results. The Journal encourages papers that lead from clearly stated purpose or rationale and from testable hypotheses, concepts, or questions to identifiable conclusions or syntheses. Such papers may amplify, modify, question, or redirect accumulated knowledge embodied in contemporary perceptions of a particular state of aquatic sciences. The Journal discourages papers that are essentially descriptive, except in emerging disciplines; have only site-specific or local applications (a certain year, place, taxon, chemical compound, etc.); only confirm previously established principles; or apply standard techniques without breaking new methodological ground.

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CJFAS has had a long tradition of publishing the proceedings of symposia in special issues and supplements of the Journal. We would like to inform our readers that this practice has not changed and to remind you of the basic guidelines for the publication of these collections of manuscripts.

Organizers of symposia should contact the Editors well in advance of the symposium date, detailing such information as the theme and scope of the symposium, as well as the expected number of contributions. A typical supplement contains 20–25 manuscripts, though larger groups of papers are acceptable. Proceedings based on fewer papers may be published as a group, along with other peer-reviewed manuscripts, in a regular issue of the Journal. Once the Editors are in general agreement that the collection of manuscripts meets CJFAS Editorial policy, symposia organizers will need to make financial arrangements with NRC Research Press. Depending upon the subject and deemed importance to the readers of CJFAS, there may be an opportunity to waive page charges partially or in entirety.

Symposium proceedings are peer reviewed with the same degree of rigor as regular Journal submissions. For groups of papers greater than 10–12, a common practice is to invite the symposia organizers to act as Guest Associate Editors during the review process. The Journal office typically handles the initial stages of the review process involving the acquisition of reviews. Although Guest Associate Editors are required to suggest the names of 2–3 potential referees per manuscript, the final selection of referees remains with the Editors. Guest Associate Editors will be asked to evaluate the reviews of each manuscript and make any additional comments, as they deem necessary. This evaluation of reviews includes the arbitration of divergent reviews, should the referees differ in their opinion of a particular manuscript. After peer review has been completed, the Guest Associate Editors are asked to correspond directly with the authors, and they are responsible for the coordination of revisions. Only when the manuscript is in final, publishable form do they return the works to the Editorial office.

For further details on CJFAS supplements and special issues, and for a complete set of guidelines, please contact the Editorial office.

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La publication de numéros spéciaux et de suppléments

Le JCSHA a l'habitude de publier les actes des symposiums dans des numéros spéciaux et des suppléments. Nous voulons aviser nos lecteurs que cette pratique n'a pas changé et vous rappeler les lignes directrices de base concernant la publication de cette collection de manuscrits.

On demande aux organisateurs du symposium de communiquer avec les éditeurs bien avant la tenue du symposium, afin de les informer du thème et de la portée de celui-ci, ainsi que du nombre de communications prévues. Habituellement, un supplément contient de 20 à 25 manuscrits, mais il est possible d'augmenter ce nombre. On peut regrouper un plus petit nombre de manuscrits et les publier avec d'autres manuscrits examinés par les pairs dans un numéro ordinaire. Quand les éditeurs conviennent que les manuscrits correspondent à la politique éditoriale du JCSHA, les organisateurs du symposium doivent conclure un accord financier avec les Presses scientifiques du CNRC. Selon le sujet et son importance pour les lecteurs du JCSHA, on pourra supprimer partiellement ou entièrement les coûts par page.

Les actes du symposium sont examinés par les pairs aussi rigoureusement que le sont les autres textes soumis au Journal à des fins de publication. Lorsque le nombre de communications groupées dépasse 10 ou 12, on invite habituellement les organisateurs du symposium à jouer le rôle de rédacteur adjoint invité pendant l'examen. Le Journal s'occupe des premières phases du processus, liées à la réalisation des examens. On demande aux rédacteurs adjoints invités de suggérer le nom de deux ou trois lecteurs par manuscrit, mais le choix final des lecteurs revient aux éditeurs. Les rédacteurs adjoints invités devront évaluer les examens effectués pour chaque manuscrit et faire des commentaires supplémentaires s'il le juge nécessaire. L'évaluation des examens comprend l'arbitrage des divergences lorsque les lecteurs n'ont pas la même opinion au sujet d'un manuscrit. Quand l'examen par les pairs est terminé, les rédacteurs adjoint invités communiquent directement avec les auteurs et coordonnent l'apport des corrections. Ils ne remettront au bureau de la rédaction que la version finale des manuscrits, prête à être publiée.

Pour avoir des renseignements supplémentaires sur les numéros spéciaux et les suppléments du JCSHA ou pour obtenir les lignes directries complètes, veuillez communiquer avec le bureau de la rédaction.

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The Second International Symposium on Krill

We are pleased to announce the Second International Symposium on Krill, **23–27 August 1999** on the campus of the University of California Santa Cruz.

The purpose of this symposium is to review current knowledge of krill euphausiid biology, to highlight gaps and future areas for research, and to show how the study of a range species of krill can illuminate more general problems (for example, krill flux, recruitment, swarming behaviour, age, and growth). The sessions (and invited speakers) are

- **Krill demography, life history, and genetic diversity** (V. Siegel, Germany; Vassily Spirodonov, Germany; T. Paternello, Italy; E. Pakamov, South Africa)
- **Krill development, growth, reproduction, and aging** (N. Iguchi, Japan; S. Nicol, Australia; J. Cuzin-Roudy, France)
- **Krill physiology, metabolism nutrition, and energetics** (L. Quetin, U.S.A.; F. Buchholz, Germany; R. Perissinoto, South Africa; Y. Nishino, Japan; P. Mayzaud, France; S. Falk-Petersen, Norway; A. Clarke, U.K.)
- **Krill behavior: swarming, vertical migration, foraging, and antipredator mechanisms** (W. Hamner, U.S.A.; D. Ritz, Australia; L. Madureira, Brazil; J Watkins, U.K.; A. Brierley, U.K.)

The opening talk will be given by D. Miller (South Africa).

Each day will include invited and contributed talks and poster sessions. Proceedings of the meeting will be published; contributors will be expected to arrive with disk and hard copies of their papers.

A call for contributed talks will occur in fall 1998. Special support will be available for young investigators and students.

Further communication will occur electronically, either at the web site **http://www2.ucsc.edu/people/msmangel/Krill_Symposium.html** or by contacting Marc Mangel by e-mail (msmangel@cats.ucsc.edu).

International Organizing Committee: Marc Mangel, Steve Nicol, Janine Cuzin-Roudy, Yoshi Endo, Denzil Miller, Robin Ross, Völker Siegel, and Jon Watkins

Local Host Committee: Sarah Barnett, Don Croll, Marc Mangel, and Baldo Marinovic

Le deuxième symposium international sur le krill

Nous sommes heureux d'annoncer la tenue du deuxième symposium international sur le krill, **du 23 au 27 août 1999,** sur le campus de l'Université de Californie, Santa Cruz.

Le symposium a pour objet d'examiner les connaissances actuelles sur la biologie du krill, de souligner les lacunes et les futurs sujets de recherche et de montrer comment l'étude de diverses espèces de krill peut éclairer des problèmes plus généraux (par exemple le flux, le recrutement, l'essaimage, l'âge et la croissance du krill). Voici les titres des exposés, accompagnés des noms des conférenciers invités.

- **Démographie, cycle biologique et diversité génétique du krill** (V. Siegel, Allemagne; Vassily Spirodonov, Allemagne; T. Paternello, Italie; E. Pakamov, Afrique du Sud)
- **Développement, croissance, reproduction et vieillissement du krill** (N. Iguchi, Japon; S. Nicol, Australie; J. Cuzin-Roudy, France)
- **Physiologie, métabolisme, nutrition et énergétique du krill** (L. Quetin, É.-U.; F. Buchholz, Allemagne; R. Perissinoto, Afrique du Sud; Y. Nishino, Japon; P. Mayzaud, France; S. Falk-Petersen, Norvège; A. Clarke, R.U.)
- **Comportement du krill: essaimage, migration verticale, quête de nourriture et protection contre les prédateurs** (W. Hamner, É.-U.; D. Ritz, Australie; L. Madureira, Brésil; J. Watkins, R.U.; A. Brierley, R.U.)

M. D. Miller (Afrique du Sud) prononcera le discours d'ouverture.

Des communications sollicitées, des communications offertes et des communications affichées seront présentées chaque jour. Les actes du symposium seront publiés; c'est pourquoi l'on demande aux conférenciers de copier leur communication sur disquette et sur copie papier.

À l'automne 1998, on lancera une invitation aux personnes intéressées à présenter des communications offertes. Un soutien spécial sera accordé aux jeunes chercheurs et aux étudiants.

À l'avenir, les renseignements seront transmis par voie électronique, soit par l'intermédiaire du site Web **http://www2.ucsc.edu/people/msmangel/Krill_Symposium.html** soit par courrier électronique (Marc Mangel : msmangel@cats.ucsc.edu).

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Estimation of fishing and natural mortality from tagging studies on fisheries with two user groups[1]

Elizabeth N. Brooks, Kenneth H. Pollock, John M. Hoenig, and William S. Hearn

Abstract: We present generalizations of fishery models that allow for the separate estimation of fishing mortality when more than one user group is present (e.g., a commercial and a recreational fishery). This model also allows for the fisheries to be in operation for any length of time whereas previously fisheries were generally considered to be pulse or continuous. Three cases are considered: (*i*) fisheries operate consecutively, (*ii*) fisheries overlap for a part of their seasons, and (*iii*) fisheries are in operation for the whole year. The results of a simulation study are included, which provide estimates of fishing and natural mortality along with their proportional standard errors (CVs). All scenarios had good precision, with most CVs < 25% and usually very little difference between the three cases. Coefficients of interaction, the potential gain by one fishery if another is closed down, are also given along with a method for calculating them. Factors affecting these coefficients of interaction were the order in which fisheries operated, amount of overlap in fishing seasons, and intensity of fishing effort by each fishery. We believe that these models could provide useful information for the management of fisheries with multiple user groups where allocation conflicts may arise.

Résumé : Nous présentons des généralisations de modèles halieutiques qui permettent l'estimation séparée de la mortalité par pêche quand il y a plus d'un groupe d'utilisateurs (pêche commerciale et pêche sportive). Ce modèle peut aussi prendre en considération des pêches de toute durée, tandis qu'antérieurement, on considérait généralement des pêches soit pulsatoires soit continues. Trois cas ont été considérés : (*i*) pêches consécutives, (*ii*) pêches chevauchantes durant une partie de leurs saisons et (*iii*) pêches qui ont lieu durant toute l'année. Nous présentons les résultats d'une étude de simulation qui fournit des estimations de la mortalité naturelle et de la mortalité par pêche avec leurs erreurs-types proportionnelles (CV). Nous avons obtenu une bonne précision dans tous les scénarios, la plupart des CV étant inférieurs à 25% et les différences entre les trois cas étant généralement très faibles. Nous présentons aussi les coefficients d'interaction, soit le gain potentiel d'une des pêches si l'autre est fermée, de même qu'une méthode pour les calculer. Les facteurs affectant ces coefficients d'interaction étaient l'ordre dans lequel les pêches étaient effectuées, le degré de chevauchement des saisons de pêche et l'intensité de l'effort de pêche dans chacune des pêches. Nous croyons que ces modèles peuvent fournir des informations utiles en vue de la gestion des pêches à groupes d'utilisateurs multiples dans lesquelles des conflits touchant les allocations peuvent survenir.

[Traduit par la Rédaction]

Introduction

Brownie et al. (1985) presented and summarized new and existing models for estimating survival rates in multiyear bird banding studies. These models have been extended to fisheries work in various formats since the 1970s, some of which permit the estimation of natural and fishing mortality in addition to yearly survival rates, provided an estimate of reporting rate is available (Youngs 1972; Youngs and Robson 1975; Jagielo 1991; Larson et al. 1991; Pollock et al. 1991; Dorazio 1993; Schwarz et al. 1993). Methods have also been introduced (Pollock et al. 1991) that allow for the estimation of instantaneous rates of mortality, as defined by Ricker (1975). However, to write down the expressions for the cells of the tagging/recovery matrix, one has to assume that a fishery is either Type I (pulse) or Type II (continuous). Hoenig et al. (1998*a*) have extended these models to include the use of effort as an auxiliary variable and also to generalize the pattern of fishing within the year. In another adaptation, Hoenig et al. (1998*b*) extended the models further to allow for nonmixing for all or part of the year of tagging.

These models were developed for one user group, although it may sometimes be the case that more than one user

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Table 1. Matrix of expected recoveries for two user groups, commercial and recreational, under Brownie Model 1 for a tagging study with three tagging years and three recovery years.

Year tagged	No. tagged	Expected recoveries Year 1	Year 2	Year 3	Fishery type
1	N_1	N_1f_{11}	$N_1S_1f_{12}$	$N_1S_1S_2f_{13}$	Commercial
		N_1f_{21}	$N_1S_1f_{22}$	$N_1S_1S_2f_{23}$	Recreational
2	N_2		N_2f_{12}	$N_2S_2f_{13}$	Commercial
			N_2f_{22}	$N_2S_2f_{23}$	Recreational
3	N_3			N_3f_{13}	Commercial
				N_3f_{23}	Recreational

group is exploiting a given fish population, e.g., a commercial fishery and a recreational fishery. In this scenario, one would like to know what proportion of the total exploitation was due to each user group. This information would be valuable in terms of setting catch limits and designating fishing seasons for each user group. It could also be used to calculate an expected gain by one fishery if the other is mandated to reduce its catch or close down completely.

In addition to being able to generalize the original models to include more than one user group, one would also like to be able to generalize the type of exploitation to describe fishing seasons that do not fit the restrictive categories of pulse or continuous (Ricker 1975). It does not seem realistic to assume that all fisheries could be classified as either strictly pulse or strictly continuous. The timing of the two fisheries will have important implications for the form of the model.

We begin with a review of the statistical nature and notation of these models before discussing generalizations of the models for the two types of fisheries. Next, we present methods for separately estimating exploitation rates when there are two user groups. We initially consider the simpler case where only slow-growing adult fish are tagged; later, we present the modifications necessary for the case where growth is not negligible. In the former case, we assume that the catchability and natural mortality of the tagged fish do not depend on their age. We also consider gains if one fishery is closed by calculating coefficients of interaction. (While we use tagging data to look at interaction, Beverton and Holt (1957) considered yield per recruit (YPR) to investigate the consequences of various management strategies in a two user group continuous fishery. The YPR approach has been extended and applied in later papers, e.g., Murawski (1984) and Shepherd (1988). In another example, Horwood and Nicholson (1991) analyzed tagging data and catch information of Dover sole (*Microstomus pacificus*) that was harvested by fleets of three countries.) Next, we present an artificial example and a simulation study. Finally, we present a general discussion section that includes suggestions for future research.

Theoretical model development

Notation

We use the following notation: S_i is the finite annual survival rate or the probability of surviving year i; u_{ji} is the finite annual exploitation rate for fishery j (j = 1 or 2) or the probability of being harvested by fishery j in year i (note that u_{ji} can be viewed as the fraction of the fish present at the beginning of year i that is harvested by fishery j); λ_{ji} is the tag-reporting rate for fishery j in year i or the probability that a tag will be found and reported to the fisheries biologist, given that the tagged fish has been harvested by fishery j; $v_i = 1 - S_i - u_{1i} - u_{2i}$ is the finite natural mortality rate or the probability of dying from a natural cause in year i in the presence of fishing mortality; $f_{ji} = \lambda_{ji}u_{ji}$ is the tag-recovery rate or the probability of a tagged fish being harvested by fishery j and reported during year i; F_{ji} is the instantaneous fishing mortality rate (expressed on a yearly basis) for fishery j in year i; M_i is the instantaneous natural mortality rate for year i; and $Z_i = F_{1i} + F_{2i} + M_i = -\log_e(S_i)$ is the instantaneous total mortality rate for year i.

Model development

Our models are developed from the same statistical framework as those of Brownie et al. (1985). Those models can be used to estimate for data that have a multinomial distribution. The multinomial distribution describes data that have more than two outcomes (the binomial distribution, which has precisely two outcomes, is a special, well-known case of the multinomial). Tag- or band-recovery data are ideally fit to a multinomial distribution, since a tagged animal can either be caught or not caught in each year after it has been initially tagged. For k years after an animal has been tagged, there are $k + 1$ possible outcomes: there is a probability that it is caught in one of the k years, which gives k outcomes; the last probability is that it is not caught during the k years, and this is 1 – (sum of all probabilities of being captured).

There are many computer programs available that are capable of estimating the parameters specified in tag-return models. Most of these programs use maximum likelihood estimation (MLE) to estimate the parameters. MLE is an estimation procedure that finds the "most likely" estimates of parameters based on the observed data.

Of the models presented in Brownie et al. (1985), Model 1 refers to "well-mixed" populations, which means that newly tagged and previously tagged animals have the same recovery rate. For the generalization of Brownie's Model 1 to two user groups (e.g., commercial and recreational), the matrix of expected recoveries for a study with three tagging years and three recovery years is shown in Table 1. For this model, newly tagged and previously tagged fish have the same recovery rate in a given year. It would be possible to generalize to allow for nonmixing in the year of tagging along the lines of Hoenig et al. (1998*b*), but for simplicity of presentation, that case is not considered here.

It is possible to estimate the quantities f_{11}, f_{21}, f_{12}, f_{22}, f_{13}, f_{23}, S_1, and S_2. Following Brownie et al. (1985), the likelihood can be constructed by treating the rows of the recovery matrix as independent multinomial distributions with the expectations as in Table 1 (cf. Schwarz et al. 1988). Expressed in terms of the finite parameters f and S, Table 1 is the familiar poststratification band-recovery formulation (Schwarz et al. 1988). We use this formulation as a starting point for the development of our models, which will permit the reexpression of these finite rates in instantaneous terms of M and user-specific Fs.

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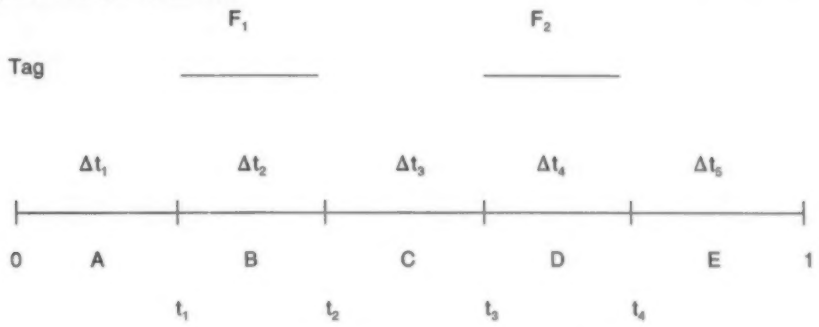
Fig. 1. Generalized scenario for two user groups (1 and 2) with nonoverlapping seasons. The length of each subinterval A, B, C, D, and E is Δt_1, Δt_2, Δt_3, Δt_4, and Δt_5, respectively.



When an estimate of reporting rate, λ_{ji}, is available for each user group, it is then possible to obtain estimates of u_{1i} and u_{2i} (i = 1, 2, 3) by interpreting f_{ji} to be the probability a tagged fish is caught multiplied by the probability that the tag is reported given that the fish is caught, i.e., $f_{ji} = u_{ji}\lambda_{ji}$. An estimate of the reporting rate can be obtained through the use of high-reward tags, planted tags, or port/creel sampling (Pollock et al. 1991).

The following seven assumptions are required for the Brownie models: (*i*) the tagged sample is representative of the target population; (*ii*) there is no tag loss, or it can be accounted for by double tagging; (*iii*) survival rates are not influenced by tagging; (*iv*) the year of tag recovery is correctly tabulated; (*v*) the fate of each tagged fish is independent of the fate of other tagged fish; (*vi*) all tagged fish within a tagged cohort have the same annual survival and recovery probabilities in a given year; and (*vii*) the survival and recovery probabilities do not depend on the age of the animal (we relax this assumption later). In addition, we make the following assumptions for our models: (*viii*) the forces of instantaneous natural and fishing mortality (M and F) are additive and independent; (*ix*) natural mortality is constant within a year (no seasonal variation) and between years; (*x*) fishing mortality for a user group is constant for the period of the year that the fishery is operating; and (*xi*) tagging takes place over a short period. For a discussion of these assumptions as they relate to fish populations, see Pollock et al. (1991).

Generalization of pulse fishery: nonoverlapping seasons

Here, we present a generalization of a pulse fishery with two user groups, as pictured in Fig. 1. The user groups have nonoverlapping periods of exploitation.

For a given year (which is represented as an interval from t = 0 to t = 1, with tagging of fish occurring at time t = 0), we can write expressions for the us and Ss as follows:

$$u_1 = S_A\left(\frac{F_1}{F_1 + M}\right)(1 - e^{-(F_1+M)\Delta t_2})$$

$$u_2 = S_A S_B S_C\left(\frac{F_2}{F_2 + M}\right)(1 - e^{-(F_2+M)\Delta t_4})$$

where S_A, S_B, and S_C are the survival probabilities for periods A, B, and C, respectively, and

$$S_A = e^{-M\Delta t_1}$$

$$S_B = e^{-(F_1+M)\Delta t_2}$$

$$S_C = e^{-M\Delta t_3}$$

$$S_{TOTAL} = e^{-(F_1\Delta t_2 + F_2\Delta t_4 + M)}.$$

Notice that this formulation of competing risks follows directly from assumptions *viii*, *ix*, and *x* (see also Youngs 1972). The total exploitation, u_{TOTAL}, for both user groups is then

$$u_{TOTAL} = u_1 + u_2.$$

This formulation allows all equations to be expressed in terms of instantaneous fishing and natural mortality rates for which we can obtain MLEs by constructing multinomial likelihoods. That is, since each cell in the recovery matrix can now explicitly be expressed in terms of F and M, one can use the program SURVIV (White 1983) to get the MLEs of F_{ji} and M. SURVIV is specifically designed for estimating multinomial likelihoods. (Note that although we estimate only one M for all three years, it is possible to estimate two separate Ms but not all three; since at least two Ms must be equal, we decided to fix M to be constant for all 3 years.)

Generalization of pulse fishery: overlapping seasons

In Fig. 2, we have two user groups whose periods of exploitation overlap for part of the fishing season. Expressions for the us and Ss are

$$u_1 = S_A(1 - S_B)\frac{F_1}{F_1 + M} + S_A S_B(1 - S_C)\frac{F_1}{F_1 + F_2 + M}$$

$$u_2 = S_A S_B(1 - S_C)\frac{F_2}{F_1 + F_2 + M} + S_A S_B S_C(1 - S_D)\frac{F_2}{F_2 + M}$$

where

$$S_A = e^{-M\Delta t_1}$$

$$S_B = e^{-(F_1+M)\Delta t_2}$$

$$S_C = e^{-(F_1+F_2+M)\Delta t_3}$$

$$S_D = e^{-(M+F_2)\Delta t_4}$$

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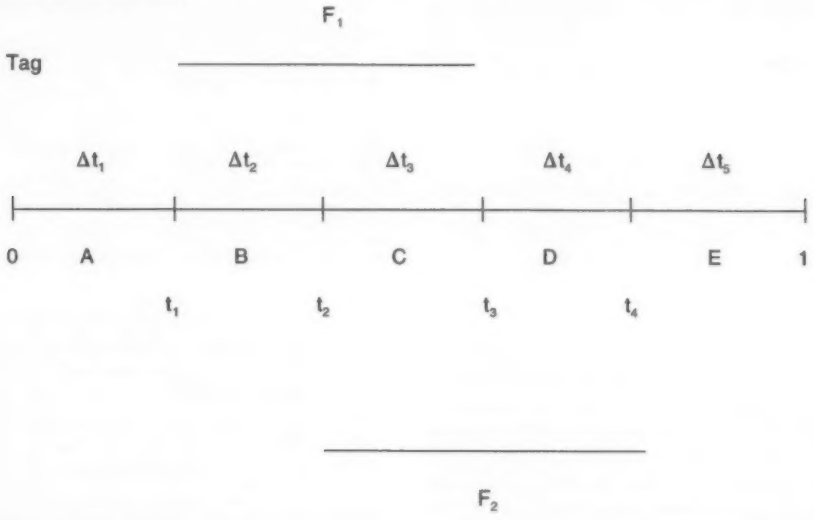
Fig. 2. Generalized scenario for two user groups (F_1 and F_2) with overlapping seasons. The length of each subinterval A, B, C, D, and E is Δt_1, Δt_2, Δt_3, Δt_4, and Δt_5, respectively.



$$S_{\text{TOTAL}} = e^{-[F_1(\Delta t_2+\Delta t_3)+F_2(\Delta t_3+\Delta t_4)+M]}.$$

The total exploitation is

$$u_{\text{TOTAL}} = u_B + u_C + u_D = u_1 + u_2.$$

(Note that u_B, u_C, and u_D are exploitations during periods B, C, and D, respectively.)

We can easily collapse this scenario into that of a pulse (Type I) fishery. If we first consider the case where we let t_3 approach ($\rightarrow$) t_2, then what we have is two user groups with no overlap, as previously considered. The total exploitation then becomes

$$u_{\text{TOTAL}} = u_B + u_D.$$

Furthermore, letting $t_1 \rightarrow t_2$ and $t_4 \rightarrow t_3$, we have

$$u_1 = e^{-Mt_1}(1 - e^{-(F_1+F_2+M)\Delta t_3})\left(\frac{F_1}{F_1+F_2+M}\right)$$

$$u_2 = e^{-Mt_1}(1 - e^{-(F_1+F_2+M)\Delta t_3})\left(\frac{F_2}{F_1+F_2+M}\right)$$

Next, allowing $\Delta t_3 \rightarrow 0$, exploitation periods become small enough that essentially no natural mortality occurs. The total exploitation can then be written

$$u_{\text{TOTAL}} = e^{-Mt_1}(1 - e^{-(F_1'+F_2')})$$

where F_1' and F_2' are the finite limiting values of $F_1\Delta t_3$ and $F_2\Delta t_3$ as F_1 and $F_2 \rightarrow \infty$ and $\Delta t_3 \rightarrow 0$. These finite limiting values can be interpreted by imagining a fishery with a season that lasts Δt_3. As that season gets shorter and shorter, the fishing intensity must increase to maintain the same harvest rate. The limiting values occur when we let the length of the season approach zero, in which case the fishing intensity in that instant is tremendous (approaching infinity). We can consider the product of the Fs and Δt_3 at these extreme values to be some fixed constant, which is denoted by F_1' and F_2'. Finally, letting $t_1 \rightarrow 0$, we are left with

$$u_{\text{TOTAL}} = 1 - e^{-(F_1'+F_2')}.$$

It should be clear from these two examples that the theory can be extended to any situation where you have two user groups simply by adjusting the Δts. For example, one can obtain expressions for two user groups that are both continuous (Type II). In Fig. 2, if we let t_1 and $t_2 \rightarrow 0$ and t_3 and $t_4 \rightarrow 1$, then we are left with the following expressions:

$$u_1 = \frac{F_1}{F_1+F_2+M}[1 - e^{-(F_1+F_2+M)}]$$

$$u_2 = \frac{F_2}{F_1+F_2+M}[1 - e^{-(F_1+F_2+M)}]$$

$$S_{\text{TOTAL}} = e^{-(F_1+F_2+M)}$$

$$u_{\text{TOTAL}} = \frac{F_1+F_2}{F_1+F_2+M}[1 - e^{-(F_1+F_2+M)}] = u_1 + u_2.$$

Clearly the theory is also easily extended to more than two user groups, but we do not consider that here.

Estimation of potential gain from closing down one fishery

Having developed the means for estimating the proportion of fishing mortality that is due to each user group, one can ask what is the effect that one group has on the catch of the other (Sibert 1984; Majkowski et al. 1988; Kleiber 1994; Bertignac 1996; Hearn and Mazanov 1996). Suppose we have two fisheries, 1 and 2, that fish the same population. Total catches by each fishery during year i are denoted C_{1i} and C_{2i}. If fishery 1 is closed for n years, then its loss in catch in numbers is $\sum_{i=1}^{n} C_{1i}$. Consequently, the gain in catch

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in numbers for fishery 2 is $\sum_{i=1}^{n} C_{2i}^{*} - \sum_{i=1}^{n} C_{2i}$, where C_{2i}^{*} is the catch by fishery 2 in the absence of fishery 1. This gain will be some proportion of the loss by fishery 1, and we call this proportion the coefficient of interaction. We can calculate the approximate expected coefficient of interaction of fishery 1 on fishery 2 (IC 1 on 2) by

$$E(\text{IC 1 on 2}) \approx \frac{E\left(\sum_{i=1}^{n} C_{2i}^{*} - \sum_{i=1}^{n} C_{2i}\right)}{E\left(\sum_{i=1}^{n} C_{1i}\right)} \quad (1)$$

which is the ratio of the expected gain in the catch of fishery 2 per unit loss of fishery 1. This assumes that the dynamics are linear, i.e., the rates of natural mortality and the fishing mortality of the continuing fishery are not affected by the closure of the other. This means that we assume that the closure of fishery 1 does not affect the catchability of fishery 2, nor does it change the ecology or the behavior of the fish or fishers. (This is satisfied if catch per unit of fishing is proportional to population numbers and the fishing effort of the remaining fishery is kept constant. Dramatic increases, however, could be limited by processing and carrying capacity of fishery 2.) In eq. 1, the expectations of the summations are simply the summations of the expectations. We can express these summations in terms of us and Ss as found in the analysis of the recovery matrix:

$$E\left(\sum_{i=1}^{n} C_{1i}\right) = P_1 \sum_{i=1}^{n} u_{1i} \prod_{k=0}^{i-1} S_{\text{TOTAL},k}$$

$$E\left(\sum_{i=1}^{n} C_{2i}\right) = P_1 \sum_{i=1}^{n} u_{2i} \prod_{k=0}^{i-1} S_{\text{TOTAL},k}$$

$$E\left(\sum_{i=1}^{n} C_{2i}^{*}\right) = P_1 \sum_{i=1}^{n} u_{2i}^{*} \prod_{k=0}^{i-1} S_{\text{TOTAL},k}^{*}$$

where $S_{\text{TOTAL},0} = 1$ and P_1 is the cohort population at the beginning of year 1. However, notice that P_1 is not needed to calculate an estimate for the interaction (eq. 1) because it cancels out of the ratio. This is significant because it is through the use of a tagging study (and the expectations for catch that are associated with it) that we are able to eliminate P_1 in eq. 1. Thus, we can estimate $E(C_{2i}^{*})$, the potential catch in year i by fishery 2 given that fishery 1 is closed for all i years, by rewriting the us and Ss in terms of Fs and Ms according to the derivations from the previous section.

The interaction coefficient is in terms of numbers of fish. This may be of interest if growth is negligible. However, the calculations can be modified to allow for growth between the times of fisheries 1 and 2. Thus, eq. 1 can be replaced by

$$E(\text{IC 1 on 2}) \approx \frac{w_{2i} E\left(\sum_{i=1}^{n} C_{2i}^{*} - \sum_{i=1}^{n} C_{2i}\right)}{w_{1i} E\left(\sum_{i=1}^{n} C_{1i}\right)}$$

where w_{2i} and w_{1i} are the mean weights in fisheries 2 and 1, respectively, in year i. These weights can be determined empirically by sampling the catches in the fisheries. Notice that we take $w_{21}^{*} = w_{21}$. This is true when fishery 1 catches fish of a younger age than fishery 2 and there is no overlapping ages. In general, however, $w_{21}^{*} > w_{21}$.

Alternatively, the calculations can be done using a YPR model, since the interaction coefficients are essentially an analysis of the YPR under two conditions. The tagging data allow one to estimate all of the necessary parameters including natural mortality and growth. If ages cannot be assigned at the time of tagging, then relative ages can be determined a posteriori from the increments in size (e.g., Fabens 1965). For the calculation of the interaction coefficients, it does not matter if the ages are absolute or shifted by a constant number of years.

Referring back to the sample recovery matrix from the model development section where we had three tagging years and three recapture years, we would be able to estimate F_{1i}, F_{2i}, and M_i for $i = 1, 2$. Assuming $M_3 = M_2$, then we can also estimate F_{13}, F_{23}, and $S_{\text{TOTAL},3}$. If the tagging experiment does not proceed past $i = 3$ years, then one may assume that conditions for years >3 would be the same as in year 3 and so a geometric progression could be summed:

$$E\left(\sum_{i=1}^{n} C_{1i}\right) = EP_1 \times \left(u_{11} + u_{12} S_{\text{TOTAL},1} + u_{13} S_{\text{TOTAL},1} S_{\text{TOTAL},2} \frac{1 - S_{\text{TOTAL},3}^{n-2}}{1 - S_{\text{TOTAL},3}}\right)$$

with similar expressions for $E\left(\sum_{i=1}^{n} C_{2i}\right)$ and $E\left(\sum_{i=1}^{n} C_{2i}^{*}\right)$. If the tagging experiment continues for longer than 3 years, say to 4 years, then F_{14}, F_{24}, and $S_{\text{TOTAL},4}$ are estimable provided M_4 is known (e.g., $M_4 = M_3 = M_2$).

The expressions for $E(u_{2i}^{*})$ and $E(S_{\text{TOTAL},i}^{*})$ can be estimated by putting $F_{1i} = 0$ into the equations for u_{2i} and $S_{\text{TOTAL},i}$. Consequently, the coefficient of interaction of fishery 1 upon fishery 2 can be estimated by analyzing a suitably designed and executed tag-return experiment without any commercial catch or effort statistics.

Age-structured models

In general, fisheries target a mixture of age groups including some that are still actively growing. The fishing mortality rates of actively growing cohorts probably change as the cohorts become older and larger, e.g., because of net size selectivity. Brownie and Robson (1976) showed that it is possible to estimate age- and year-specific survival rates from

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Table 2. Age-structured matrix of expected recoveries for two user groups for a tagging study with three tagging years and three recovery years.

Year tagged	No. tagged	Expected recoveries			Fishery type
		Year 1	Year 2	Year 3	
Fish tagged and released at age a					
1	N_{1a}	$N_{1a}f_{11a}$	$N_{1a}S_{1a}f_{12a+1}$	$N_{1a}S_{1a}S_{2a+1}f_{13a+2}$	Commercial
		$N_{1a}f_{21a}$	$N_{1a}S_{1a}f_{22a+1}$	$N_{1a}S_{1a}S_{2a+1}f_{23a+2}$	Recreational
2	N_{2a}		$N_{2a}f_{12a}$	$N_{2a}S_{2a}f_{13a+1}$	Commercial
			$N_{2a}f_{22a}$	$N_{2a}S_{2a}f_{23a+1}$	Recreational
3	N_{3a}			$N_{3a}f_{13a}$	Commercial
				$N_{3a}f_{23a}$	Recreational
Fish tagged and released at age $a + 1$					
1	N_{1a+1}	$N_{1a+1}f_{11a+1}$	$N_{1a+1}S_{1a+1}f_{12a+2}$	$N_{1a+1}S_{1a+1}S_{2a+2}f_{13a+3}$	Commercial
		$N_{1a+1}f_{21a+1}$	$N_{1a+1}S_{1a+1}f_{22a+2}$	$N_{1a+1}S_{1a+1}S_{2a+2}f_{23a+3}$	Recreational
2	N_{2a+1}		$N_{2a+1}f_{12a+1}$	$N_{2a+1}S_{2a+1}f_{13a+2}$	Commercial
			$N_{2a+1}f_{22a+1}$	$N_{2a+1}S_{2a+1}f_{23a+2}$	Recreational
3	N_{3a+1}			$N_{3a+1}f_{13a+1}$	Commercial
				$N_{3a+1}f_{23a+1}$	Recreational
Fish tagged and released at age $a + 2$					
1	N_{1a+2}	$N_{1a+2}f_{11a+2}$	$N_{1a+2}S_{1a+2}f_{12a+3}$	$N_{1a+2}S_{1a+2}S_{2a+3}f_{13a+4}$	Commercial
		$N_{1a+2}f_{21a+2}$	$N_{1a+2}S_{1a+2}f_{22a+3}$	$N_{1a+2}S_{1a+2}S_{2a+3}f_{23a+4}$	Recreational
2	N_{2a+2}		$N_{2a+2}f_{12a+2}$	$N_{2a+2}S_{2a+2}f_{13a+3}$	Commercial
			$N_{2a+2}f_{22a+2}$	$N_{2a+2}S_{2a+2}f_{23a+3}$	Recreational
3	N_{3a+2}			$N_{3a+2}f_{13a+2}$	Commercial
				$N_{3a+2}f_{23a+2}$	Recreational

Note: To separate age and year effects, all ages must be tagged in all years. For this study, age-specific recovery rate can be estimated for ages a, $a + 1$, and $a + 2$ for years 1, 2, and 3. Age-specific survival can be estimated for ages a and $a + 1$ for years 1 and 2.

tagging data if the ages of the fish are determined at the time of tagging.

Consider the case where fish of age a, $a + 1$, and $a + 2$ are tagged each year and recoveries are recorded according to the age of initial tagging and release (see Brownie et al. 1985, p. 119). It can be seen from the table of expected recoveries (Table 2) that it is possible to estimate survival rates. For example, the survival rate of age a animals in year 1, S_{1a}, can be estimated from the ratio of returns for a given fishery:

$$\hat{S}_{1a} = \frac{R_{12a}}{R_{22a+1}} \times \frac{N_{2a+1}}{N_{1a}}$$

where R_{12a} are the tags returned in year 2 of fish tagged in year 1 at age a and R_{22a+1} are the tags returned in year 2 of fish tagged in year 2 at age $a + 1$. (Here, the returns could refer to returns from the commercial fishery, the recreational fishery, or both combined.) Similarly, the survival rate of age $a + 1$ animals in year 2 can be estimated by looking at the ratio of recoveries of animals tagged in years 2 and 3. It is clear that survival rates for all ages and all years (except the last age and the last year) can be estimated if all ages are tagged in all years.

As in the case without age structure, we can replace the Ss with functions of the (now age-specific) Fs and M as previously derived; also, we can replace the fs with products of $u\lambda$ and the us with functions of the (age-specific) Fs and M. Thus, each cell in the series of recovery matrices can be associated with a cell probability that is a function of the natural mortality rate, tag-reporting rate, and the series of fishing mortality rates that has been experienced by the cohort. The fishing mortality rates can be age, year, and user group specific. Depending on the data, the natural mortality rate can be made variable to some extent over age or time (see Hoenig et al. 1998*a*). Likewise, tag-reporting rates can be specified as variable if sufficient data are available to estimate each reporting rate.

Interaction coefficients can be calculated from the resulting estimates of fishing and natural mortality. The situation is, however, more complicated than for the non-age-structured case because exploitation varies both by age and time, rather than just by time. Consider first the immediate effects of the closure of fishery 1. The approximate expected value of the interaction coefficient in the year of closure is

$$E(\text{IC 1 on 2}) \approx \frac{\sum_{a=1}^{A} E(C_{1a})u^*_{2a}}{\sum_{a=1}^{A} E(C_{1a})}$$

where u^*_{2a} is the exploitation rate for fishery 2 in the absence of fishery 1. The new exploitation rate for fishery 2 can be calculated from the fishing mortality rate for fishery 2, the natural mortality rate, and information on the timing of the fishery (the fishing mortality rate for fishery 2 does not change if fishery 1 is eliminated). Thus, of the C_{1a} fish not caught due to the closure of fishery 1, the fraction u^*_{2a} of them will be caught that year by fishery 2.

Note that it is possible that the closure of fishery 1 has no immediate effect on fishery 2. This occurs, for example, if

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Table 3. Example of a tagging/recovery matrix with three tagging years and three recovery years when a commercial and a recreational fishery are both present.

Year tagged	No. tagged	No. of recoveries Year 1	Year 2	Year 3	Fishery type
1	1000	60	45	20	Commercial
		35	25	5	Recreational
2	850		50	30	Commercial
			20	10	Recreational
3	925			45	Commercial
				15	Recreational

fishery 1 harvests 3-year-olds, while fishery 2 harvests 4-year-olds. Thus, it is of interest to determine the effect of closure over various periods of time.

The cumulative interaction after 2 years can be defined to be the increase in catch of fishery 2 over the 2-year period per unit loss of catch from fishery 1 over the 2 years. Because present catch is more valuable than future catch, one may wish to discount the annual catches by an appropriate interest rate. The expected value of the cumulative interaction coefficient (CIC) can now be defined as

$$E(\text{CIC 1 on 2 (2 years)}) \approx \frac{\sum_{a=1}^{A}\sum_{t=1}^{2} E(C_{1at})u^{*}_{2at}w_{at} + \sum_{a=1}^{A-1} E(C_{1A1})S^{*}_{a1}u^{*}_{2,a+1,2}w_{a+1,t+1}\delta}{\sum_{a=1}^{A}\sum_{t=1}^{2} E(C_{1at})w_{at}\delta^{t-1}}$$

where δ is the discounting factor that is related to the interest rate i by $\delta = (1 + i)^{-1}$. Here, the denominator is the (discounted) loss to fishery 1 by the closure. The numerator is the gain to fishery 2. It has two parts: the immediate and the delayed gain. That is, some of the fish not taken by fishery 1 (because of the closure) will be harvested in year 1 and some will be harvested in year 2. The same approach can be used to extend the formula to the cumulative interaction over a longer period of time. This formulation is a simplification, however, and does not account for costs associated with handling, processing, and distribution.

Example

For illustrative purposes, we present an artificial example of a tagging/recovery matrix. Consider the example in Table 3 with three tagging years and three recovery years. We assume that these data came from two user groups that fished continuously, so that no adjustment for time scale is necessary for the estimated Fs. Using the program SURVIV (White 1983), we can obtain the MLEs of the parameters F_{1i}, F_{2i}, M_i, S_i (i = 1, 2), u_{13}, and u_{23}. For Type II fisheries in general, it is necessary to introduce a constraint on M for the last year to be able to estimate all of the Fs. We opted to constrain all three Ms to be equal. Notice that this is not necessary for Type I fisheries, since M does not appear in the expressions for the us, and so all of the Fs and Ms are separable. This additional constraint also usually provides better precision. Accepting a constant natural mortality rate, we can now estimate F_{1i}, F_{2i} (i = 1, 2, 3), and M. We used a reporting rate of 0.5 in this example for both of the user groups. Estimates for this model are found in Table 4.

Table 4. Maximum likelihood parameter estimates for instantaneous rates of fishing (F) and natural (M) mortality rate as computed using the program SURVIV (White 1983).

Parameter	Estimate	Estimated SE
F_{11}	0.1429	0.0196
F_{21}	0.0418	0.0073
F_{12}	0.1473	0.0194
F_{22}	0.0348	0.0059
F_{13}	0.1108	0.0173
F_{23}	0.0175	0.0038
M	0.1827	0.0791
IC 1 on 2	0.0407	0.0044
IC 2 on 1	0.1852	0.0131

It was possible to estimate variances for the interaction coefficients from a single data set in the following way: (*i*) we ran SURVIV with "proc estimate" to get estimates of Fs and M, (*ii*) we then reran SURVIV with "proc simulate" and initialized all of the parameters in the model by the estimated values from step *i*, and (*iii*) we used SAS (SAS Institute Inc. 1989) "proc means" to calculate the interaction coefficients and the variance of the interaction coefficients.

Since fishery 1 had higher annual Fs, it was able to pick up a greater proportion of fishery 2's catch (~19%) whereas fishery 2 was only able to pick up about 4% of the catch if fishery 1 was closed.

In practice, data pertaining to reporting rate (as obtained from a variable-reward study, planted tags, or a port sampling program) can be included in the likelihood for the recapture matrix so that the reporting rate and mortality rates are estimated simultaneously (see Hoenig et al. 1998*a*).

Simulation study

Simulation methods

We performed a simulation study to explore the precision of parameters in our models and dynamics of the coefficients of interaction under different fishing scenarios. Three possible scenarios for fisheries operation were considered in these simulations: a continuous case, an overlap case, and a consecutive case (see Fig. 3). In the continuous case, both fisheries were assumed to be in operation for the entire year (where the time interval is 0–1 for the whole year). For the overlap case, fishery 1 operated from time t_1 = 0.25 to t_3 = 0.75, while fishery 2 operated from time t_2 = 0.50 to time t_4 = 1.0. Thus, there was overlap for 0.25 of the year. Finally, in the consecutive case, fishery 1 operated from time t_1 = 0.25 to time t_2 = 0.5 and fishery 2 operated from time t_3 = 0.5 to t_4 = 1.0.

In all three cases, we allowed the Fs to take the values 0.1, 0.2, or 0.3 per year and the Ms could be 0.2 or 0.4 per year. For scenarios where the fishery operated for less than 1 year, the product of $F\Delta t$ (where Δt is the fraction of the year fished) was set to the above values. Hence, a fishery that operated for half the year fished with F = 0.6 for that amount of time, which is analogous to a continuous fishery with F =

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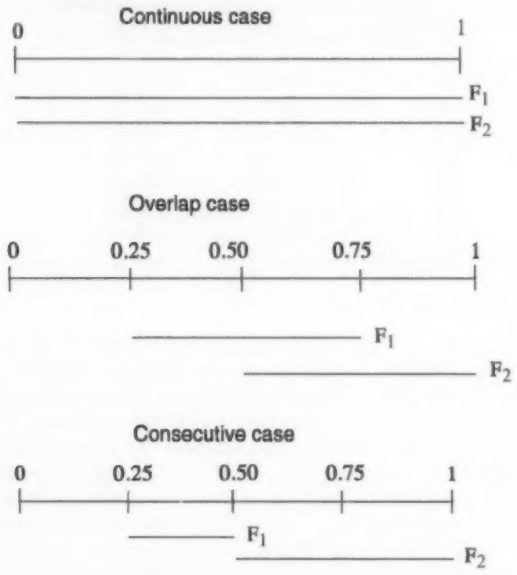
Fig. 3. Fishery scenarios considered for each simulation study. The two user groups (1 and 2) have fishing mortalities F_1 and F_2, and the length of the bars represents the time of their respective operation.



0.3. This adjustment was done so that expected tag returns would be comparable, therefore making comparisons in precision more equitable. The size of the cohorts (number tagged each year) was either 500 or 1000, and the reporting rate was fixed at $\lambda = 0.8$ or 0.5. We estimated the parameters using proc simulate and proc estimate in the program SURVIV (White 1983) with 1000 simulations for each case.

In each simulation, we estimated a full and a reduced model. In the full model, F_{1i} and F_{2i} (i = 1, 2, 3) were allowed to vary between years, but the Ms were constrained ($M_1 = M_2 = M_3$). In the reduced model, we constrained the Fs and the Ms ($F_{11} = F_{12} = F_{13}$, $F_{21} = F_{22} = F_{23}$, and $M_1 = M_2 = M_3$). It was necessary to constrain the Ms to be able to estimate F_3. Without constraining the Ms, we can only estimate u_{13} and u_{23} for the third year.

Altogether, we had 72 different runs ($3 \times 2 \times 2 \times 2$ different parameter combinations for three different fishery scenarios). Sample output is given in Table 5, where we compare the estimates and proportional standard errors, or coefficients of variation (CV), between the three scenarios.

Simulation results

Within each of the three fishing scenarios, there were consistent patterns between the CVs of the parameters. We found that CV($F = 0.1$) > CV($F = 0.2$) > CV($F = 0.3$) and also that CV($M = 0.2$) > CV($M = 0.4$). Holding the Fs constant and increasing M caused the standard error on the Fs to increase. This was true for both the full and reduced models. In general, increasing the size of the cohort (from $N = 500$ to $N = 1000$) led to greater precision for the parameter estimates, as did increasing the reporting rate (from $\lambda = 0.5$ to $\lambda = 0.8$). More precision was gained from increasing the number of tagged fish than from increasing the reporting rate.

Table 5. Sample output from 1000 simulations.

Parameter	Continuous	Overlap	Consecutive
Full model			
F_{11}	0.1021 (13%)	0.2020 (11%)	0.4038 (9%)
F_{12}	0.0977 (11%)	0.2002 (12%)	0.3998 (9%)
F_{13}	0.0982 (11%)	0.2015 (13%)	0.4029 (10%)
F_{21}	0.2961 (7%)	0.5926 (7%)	0.5925 (8%)
F_{22}	0.2950 (6%)	0.5924 (8%)	0.5928 (8%)
F_{23}	0.2954 (7%)	0.5968 (9%)	0.5964 (9%)
M	0.2053 (17%)	0.2093 (17%)	0.2089 (16%)
IC 1 on 2	0.3461 (3%)	0.4019 (4%)	0.4050 (4%)
IC 2 on 1	0.1382 (6%)	0.1133 (7%)	0.1032 (7%)
Reduced model			
F_{1i}	0.0991 (7%)	0.2009 (8%)	0.4014 (6%)
F_{2i}	0.2959 (5%)	0.5926 (5%)	0.5925 (5%)
M	0.2071 (15%)	0.2078 (14%)	0.2074 (13%)

Note: Estimates of mean of parameters (with CV in parentheses) are compared for the three scenarios, continuous, overlap, and consecutive, for full and reduced models. Results presented are for the case where $N = 1000$, $\lambda = 0.8$, and F_{1i} (or $F_{1i}\Delta t$) was initialized at 0.1, F_{2i} (or $F_{2i}\Delta t$) was initialized at 0.3, and M was initialized at 0.2.

Comparing the estimates between the three fishing scenarios, we found the precisions to be very close, with no scenario consistently having the lowest CVs. For the "best" case, where N and λ were highest ($N = 1000$ and $\lambda = 0.8$), the CVs between scenarios only differed by at most 3%. For the "worst" cases, where N and λ were lowest, the difference in CVs was about 10%. The range of CVs for best cases was around 10% for Fs and 10–20% for M, while for the worst cases, CVs were 10–25% for Fs and 15–32% for M. The reduced cases (where both F and M were constant for all three years) were more precise than the full cases. Bias was negligible for all parameters in the simulations.

The coefficients of interaction were also calculated for each simulation run (see Table 5). Overall, the interaction coefficients were more precise than the estimates of F and M and were also unbiased. The precision only changed by about 3% between the best and worst cases. There was a large difference in the coefficients when the fisheries operated at different times of the year, as in the overlap and consecutive scenarios. The fishery operating first during a given year always saw a smaller gain when the second fishery, which fished later in the year, was closed. For the consecutive case, this gain ranged from 10 to 27%, and in the overlap case the gain ranged from 10 to 30%. When the first fishery was closed, the remaining fishery in these scenarios saw a much greater gain in catch, depending on its own level of effort. In both the consecutive and overlap cases, the second fishery could realize a gain of 14–40%. These "gains" should be interpreted as the proportion of the closed fishery's catch that could potentially be caught by the remaining fishery. The higher the F of the remaining fishery, the more it will catch per unit lost by the closed fishery.

A second type of interaction coefficient could be defined that would measure the proportional change in the catch of the remaining fishery, $(C_2^* - C_2)/C_2$, i.e., this coefficient

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would inform a fishery as to how much its catch would increase in proportion to what its own total catch was when both fisheries were in operation. For the consecutive case, this coefficient ranged from 6 to 24% for the first fishery and from 17 to 68% for the second fishery, while in the overlap case the range was from 7 to 28% for the first fishery and from 16 to 61% for the second fishery. A fishery with a low F could realize a greater proportional increase in its own total catch than a fishery with a higher F.

In the continuous case, where both fisheries operated all year long, a fishery could expect to take up 12–35% of its competitor's catch. Alternatively, this would translate into increasing its own total catch by about 11–43%.

If M was increased from 0.2 to 0.4, the interaction coefficients decreased. This is because fish foregone by the closed fishery would be likely to die as a result of natural mortality before they could be caught by the remaining fishery.

Discussion

By allowing that a fishery can be in operation for any amount of time, rather than lumping into the categories of pulse or continuous, we can calculate coefficients of interaction that more accurately reflect the potential gains in catch. The timing of the fisheries is very important in determining fishing mortalities and interaction coefficients. In particular, the order in which the fisheries operate regulates the extent of the effect seen when one fishery is closed.

The coefficient of interaction, as defined in eq. 1, represents the proportion of the catch foregone by the closed fishery that will be taken up by the remaining one. This estimate could be useful to management, in terms of setting and reevaluating catch limits and season lengths. A second coefficient of interaction can be defined as $(C_2^* - C_2)/C_2$, which is an estimate of the percentage increase that a particular fishery would see. This would be of more direct interest to fisheries in terms of planning and economic strategy, as it would allow a better "guess" at manpower, gear, and vessels that would be needed should a competing fishery be closed down.

For the simulation results, based on our choices for parameters, the values of the interaction coefficients are not high, but they are estimated in terms of the number of fish caught and the closure is considered for only the duration of the tagging experiment, which was 3 years. The values will be higher for longer closures. They will also be higher if biomass is taken into account because fish forgone will be heavier (sometimes much heavier) when recaptured later by the remaining fishery, which could be a topic of further investigation. This approach would be suitable for assessing interactions where differences between users relate to differences in selectivity. For example, one fishery might catch mainly 1-year-old fish and the other fishery mainly 3-year-old fish from the same year-class. In such a situation, management might close the fishery on 1-year-olds if recruitment overfishing was a concern.

The general formulas presented were demonstrated for two user groups, although they can clearly be applied to any number of user groups and thus can be used when data on a multiple user fishery are available, provided the tag returns are recorded separately and an independent estimate of reporting rate is available for each user group (from a creel survey, port sample, reward tagging, etc.).

We have investigated some aspects of interactions among multiple-user fisheries, thus illustrating how analyses of data from a properly designed and implemented tagging experiment can be used to assess and manage such fisheries.

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Comparison, using minisatellite DNA profiling, of secondary male contribution in the fertilisation of wild and ranched Atlantic salmon (*Salmo salar*) ova

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Abstract: Secondary males, either subdominant adults or, more usually, parr which mature in freshwater, are known to successfully fertilise Atlantic salmon (*Salmo salar*) ova. This study has estimated the reproductive success of such secondary males in redds formed by pairs of wild and sea-ranched adults. Eight wild and 11 ranched redds were sampled from the Burrishoole system, western Ireland, in two consecutive years. Representative samples of fertilised ova were screened at three minisatellite loci to reconstruct the genotypes of putative anadromous parents and to estimate the proportion of eggs fertilised by secondary males. Multiple paternal genotypes were detected in 18 of the 19 samples, and of the 1484 progeny examined, 593 could not have been derived from the primary adult male genotype. The level of secondary male contribution detected among wild redds from Burrishoole (mean 28.9%) was similar to that found in previous published studies. The level among ranched redds was significantly higher (mean 48.2%) (P = 0.042). The difference was greater when data from 1995 were considered alone, with the average level of secondary male contribution in ranched redds being almost twice that in wild redds. Minimum numbers of secondary males were not significantly different between wild (mean 2.6) and ranched (mean 2.7) redds.

Résumé : Il est connu que des mâles secondaires, c'est-à-dire des adultes sous-dominants ou, plus généralement, des tacons devenus matures en eau douce, fécondent avec succès des oeufs de saumon de l'Atlantique (*Salmo salar*). La présente étude a porté sur l'estimation du succès reproductif de ces mâles secondaires dans les nids creusés par des paires d'adultes sauvages ou d'élevage par mise en valeur. Huit nids de poissons sauvages et 11 de poissons d'élevage du bassin de la Burrishoole, dans l'ouest de l'Irlande, ont été échantillonnés au cours de deux années consécutives. Des échantillons représentatifs d'oeufs fécondés ont fait l'objet d'un examen portant sur trois locus de minisatellites afin d'établir le génotype des parents anadromes présumés et d'estimer la proportion d'oeufs fécondés par des mâles secondaires. Des génotypes paternels multiples ont été décelés chez 18 des 19 échantillons, et des 1484 oeufs fécondés examinés, 593 ne pouvaient provenir du génotype du mâle adulte primaire. La valeur de l'apport des mâles secondaires déterminée pour les nids de poissons sauvages de la Burrishoole (moyenne de 28,9%) était semblable à celle mentionnée dans les études déjà publiées. La valeur était beaucoup plus élevée pour les nids de poissons d'élevage (moyenne de 48,2%) (P = 0,042). L'écart était supérieur lorsque les données de 1995 étaient évaluées seules, la valeur moyenne de l'apport des mâles secondaires pour les nids de poissons d'élevage atteignant presque le double de celle des nids de poissons sauvages. Le nombre minimum de mâles secondaires ne différait pas de façon significative entre les nids de poissons sauvages (moyenne de 2,6) ou d'élevage (moyenne de 2,7).

[Traduit par la Rédaction]

Introduction

Most European populations of Atlantic salmon (*Salmo salar*) are anadromous with the juvenile stages being spent in freshwater and the adult maturation stage in the sea. In the British Isles, juveniles, known as parr, typically spend 2 or 3 years in freshwater. After migration to sea as smolts, and following one or more winters at sea, sexually maturing adults return to spawn. As in other salmonid species, a variable proportion (up to 100%) of Atlantic salmon male parr become sexually mature without leaving freshwater (Fleming 1996). Males that mature in freshwater will be smaller than adults who experience enhanced feeding and growth at sea, although sexual maturation as parr is not thought to inhibit future smoltification and completion of the normal anadromous life cycle (Saunders et al. 1994; Fleming 1996).

The spawning behaviour of Atlantic salmon has been the subject of many studies (reviewed by Fleming 1996). During spawning, the female selects a site to deposit ova in a gravel substrate, forming a series of depressions or a redd, generally in the presence of an anadromous male. In addition, a

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number of secondary males may be present. These may include subdominant anadromous males, or more usually sexually mature parr. Despite aggression from the primary anadromous male, and to a lesser extent the female, secondary males will attempt to "sneak" into the redd and fertilise ova during the spawning event.

Sexually mature parr have commonly been observed participating in spawning events (e.g., Myers and Hutchings 1987; Fleming 1996), but only with the development of genetic markers has it been possible to accurately estimate their contribution to fertilisation of ova. The earliest studies were carried out by using spawning adults and parr with known allozyme genotypes in experimental (Hutchings and Myers 1988) or natural (Jordan and Youngson 1992) conditions. Spawning individuals were screened for variation at a single locus and preselected combinations of adults and parr used so that offspring could be assigned to adult or parr males. The use of more variable genetic markers, such as minisatellite or microsatellite variable number tandem repeat (VNTR) loci, has meant that parentage can be assigned after the spawning has taking place, precluding the need for biopsy and preselection of individuals. These techniques have been used where adult spawners were sampled by trapping (Thomaz 1995) or in experimental channels where the numbers of adults and parr were manipulated (Morán et al. 1996; Thomaz et al. 1997).

Due to their economic importance, Atlantic salmon have been cultured for commercial and stocking purposes for several decades. Intensive farming of Atlantic salmon has been demonstrated to cause phenotypic (Fleming and Jonsson 1994), genetic (e.g., Verspoor 1988), and behavioural divergence (e.g., Fleming et al. 1996, 1997) from wild populations. Sea ranching involves the culture of juveniles in hatcheries until they are released into the sea as smolts. Fish then migrate and mature at sea under the same conditions as wild fish before returning to freshwater and "homing" to their point of release.

The present study differs from previous work in that minisatellite DNA profiles of progeny are used to reconstruct the genotypes of the female and primary male participating in each redd examined. This approach relies on the typically high number of alleles observed at VNTR loci to derive parental genotypes from Mendelian expectations. The aim of this study is twofold. Firstly, we attempt to demonstrate that parental structuring of redds can be investigated with minimum environmental disturbance and without knowledge of the putative parents involved. Secondly, differences in reproductive behaviour suggest that cultured Atlantic salmon adult males may be less successful in fertilising ova. This study compares the levels of secondary male (sexually mature parr or subdominant adult) participation in the redds of wild and ranched Atlantic salmon under similar conditions.

Materials and methods

Survey and excavation of redds

The Burrishoole system in western Ireland (53°55′N, 95°55′W), with a total catchment area of over 90 km^2, consists of the freshwater Lough Feeagh and numerous afferent rivers. Lough Feeagh is connected to the marine Lough Furnace by two short outlet channels, each of which has complete upstream trapping facilities (Salmon Leap and Mill Race). The ranched stock used in this study was established from the Burrishoole wild stock in 1964. A separate ranched line has been maintained since then by the use of returning ranched adults as broodstock (Piggins and Mills 1985). All ranched smolts released are tagged using coded-wire microtags. Most ranched salmon returning to Burrishoole home to areas just upstream of the Salmon Leap and Mill Race traps, thus permitting capture of ranched individuals that are floy tagged before release upstream of the traps. Wild salmon also move through the traps, but most spawn in the afferent rivers of Lough Feeagh.

During November and December 1994 and 1995, regular surveys of selected Atlantic salmon spawning areas were carried out within the Burrishoole system. Spawning activity was observed to determine the location of redds and whether they were formed by wild or ranched salmon (Table 1). To facilitate ranched salmon attempting to spawn in the Salmon Leap and Mill Race channels, additional gravel (about 25 m^2 and at least 30 cm deep) was introduced upstream of the traps. The gravel was taken from a typical salmon spawning area of the Black River from which five of the wild redd samples were later obtained. Ranched salmon (identified by floy tags) only were observed spawning at both enhanced sites in the presence of parr. Wild salmon were observed in several sites higher in the system, including the three rivers from which redds were sampled. No floy-tagged individuals were observed in these areas. The ranched and wild redds were thus separated by Lough Feeagh and a distance of some 3 km. The positions of redds were marked and, within 24–48 h of completion, redds were covered with 25-mm wire mesh in an attempt to prevent overcutting by subsequent spawners. Redds were excavated in February 1995 and March 1996. All recoverable eyed embryos, or predispersal alevins, were preserved in 99% ethanol, and of these, a random sample was analysed.

DNA profiling

Embryos from ethanol-preserved samples were dissected free from the attached yolk sacs. DNA was extracted from whole embryos or alevins, and all samples were screened at three minisatellite (VNTR) loci: *Str*-A22/1, *Ssa*-A45/2/1, and *Str*-A9 (Prodöhl et al. 1995; Taggart et al. 1995). Two redds were analysed at an additional two loci: *Ssa*-A60 (Taggart et al. 1995) and *Ssa*-A10 (J. Taggart, University of Stirling, Stirling, Scotland, personal communication). In addition to a molecular weight marker in one lane, an internal marker system (Taggart and Ferguson 1994) was used in each lane to aid accurate typing of alleles. Differentiation between Atlantic salmon and brown trout (*Salmo trutta*) was carried out on the basis of allele size distributions and, where necessary, confirmed by amplification of 5S rDNA, which shows a size difference between the species (Pendás et al. 1995).

Mitochondrial DNA restriction fragment length polymorphism (RFLP) analyses

Mitochondrial DNA analysis was carried out as an independent check of whether an individual egg was extraneous to the redd of interest and as an aid in determining the maternal genotype. The NADH dehydrogenase-1 (ND1) region was amplified using the polymerase chain reaction (PCR) (Cronin et al. 1993) and the product digested with *Hae* III, *Rsa* I, and *Hinf* I to construct a composite haplotype.

Paternal identification and interpretation of secondary male contributions to fertilisation

The DNA profiles of offspring at three loci were used to reconstruct the most likely genotypes of the female and primary male adults. The patterns of variation were interpreted as follows.

(*i*) When more than one male contributed to the spawning, maternal alleles were determined as being the pair of alleles, one of

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Table 1. Location and date redds were covered, mean number of alleles per loci, and observed heterozygosities (H_{obs}) of progeny.

	Location	Date redd covered (day.month.year)	Redd	Mean no. of alleles per locus (±SE)	Mean H_{obs} (±SE)
Wild redds					
1995	Black River	25.11.94	A	6.3 (±0.3)	0.906 (±0.045)
	Black River	26.11.94	B	4.7 (±0.3)	0.599 (±0.088)
	Black River	26.11.94	C	3.7 (±0.3)	1.000 (±0)
	Black River	26.11.94	D	6.3 (±1.9)	0.712 (±0.032)
	Black River	14.12.94	E	3.7 (±0.7)	0.524 (±0.080)
	Rough River	16.12.94	F	6.0 (±0)	0.652 (±0.118)
	Rough River	19.12.94	G	7.0 (±1.0)	0.646 (±0.174)
1995 mean (SD)				5.4 (1.3)	0.734 (0.171)
1996	Lodge River	Unknown	H	7.0 (±1.5)	0.829 (±0.112)
1995 and 1996 mean (SD)				5.6 (1.4)	0.734 (0.163)
Ranched redds					
1995	Salmon Leap	02.12.94	A	5.7 (±0.7)	0.628 (±0.230)
	Salmon Leap	02.12.94	B	5.3 (±0.3)	0.683 (±0.010)
	Salmon Leap	02.12.94	C	4.7 (±0.7)	0.684 (±0.047)
	Mill Race	30.11.94	D	3.7 (±0.3)	0.590 (±0.051)
	Mill Race	30.11.94	E	5.7 (±0.7)	0.703 (±0.126)
1995 mean (SD)				5.0 (0.8)	0.658 (0.047)
1996	Salmon Leap	08.01.96	F	5.7 (±1.2)	0.636 (±0.232)
	Salmon Leap	08.01.96	G	4.7 (±0.3)	0.833 (±0.150)
	Salmon Leap	08.01.96	H	5.3 (±0.3)	0.649 (±0.158)
	Mill Race	08.01.96	I	5.3 (±0.3)	0.802 (±0.044)
	Mill Race	08.01.96	J	4.3 (±0.3)	0.773 (±0.111)
	Mill Race	08.01.96	H	5.7 (±0.7)	0.791 (±0.030)
1996 mean (SD)				4.8 (0.7)	0.747 (0.084)
1995 and 1996 mean (SD)				4.9 (0.7)	0.710 (0.081)

which was found in every progeny, or alternatively the single allele common to all progeny assuming the female parent to be homozygous. When more than two alleles made up the maternal genotype, it was assumed that these were extraneous eggs from another female and were excluded from analysis.

(*ii*) If a single pair of fish were involved in spawning, then there would be a maximum of four alleles per locus among the progeny, assuming both parents were heterozygous for different alleles. Additional paternal alleles at one or more of the loci were assumed to arise through the participation of a secondary male(s) in the spawning event. Alleles from the primary adult male were considered to be the two most common paternal alleles (heterozygote), or the single most common one (homozygote) when this appeared at more than twice the frequency of any other paternal allele. Therefore, it was assumed that the primary adult male contributed more than any single parr or subdominant adult male.

(*iii*) In the majority of redd samples, no single locus detected all of the secondary male progeny. Therefore, using only one locus would result in a considerable underestimation of secondary male progeny and numbers of extrapaternal alleles. While the two most common or single prevalent paternal allele can be assumed to come from a single male, the relatively low frequency of each secondary male allele means no similar assumptions can be made. For example, if eight extrapaternal alleles are found at a particular locus, these could be contributed by eight secondary males (assuming each is homozygous for a different allele) or from four secondary males (assuming 100% heterozygosity). Considering the typical heterozygosity values for these minisatellite markers, 0.82 averaged over three loci (Taggart et al. 1995), suggests that the actual number is somewhere less than 1.5 times the minimum value. In the above example, this would suggest that three of the minimum four secondary males involved would be heterozygous, accounting for six of the eight alleles, and the remaining two alleles from homozygotes, giving a total of five secondary males.

Statistical analysis

The range of values for secondary male contribution and minimum numbers of secondary males was tested for normality using the Shapiro–Wilk test, an appropriate test of normality when $N < 50$. The variances of the samples were tested using the Bartlett χ^2 test. Values with normal distributions and equal variances were tested for significant differences using Student *t*-tests; otherwise, Mann–Whitney *U*-tests were used to analyse nonparametric data.

Results

At each locus, individuals showed one (homozygote) or two (heterozygote) bands representing minisatellite alleles of different electrophoretic mobility. The range of allele sizes differed among loci from 3.35 to 10.05 kilobase pairs (kb). Most alleles were well separated and easily discriminated. However, where there was a more continuous distribution range of allele sizes, adjacent alleles were "binned" to ensure more accurate typing of genotypes. Two or more alleles were binned in the following size ranges: *Str*-A22/1, 4.45–4.65 kb; *Ssa*-A45/2/1, 7.65–7.81 kb. This, combined with

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Fig. 1. Autoradiograph showing 32 individuals from an Atlantic salmon redd (ranched redd C) screened at locus *Str*-A22/1. Putative maternal and primary male alleles are denoted by arrows. *Eco* RI/*Hin*d III digested λ DNA is shown on the right with molecular weights in kilobase pairs.

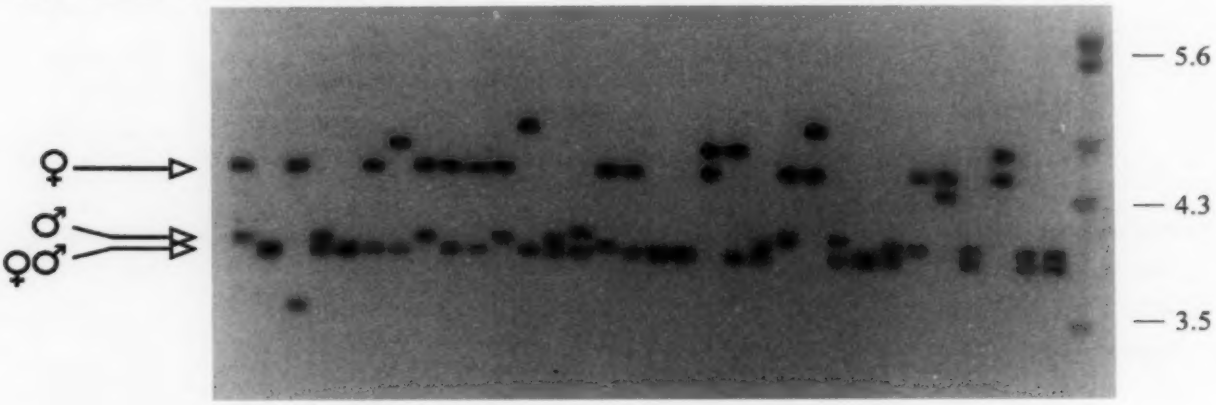

Table 2. Summary of genetic analysis of redds formed by wild Atlantic salmon in the Burrishoole system.

Redd	% extraneous eggs	N (excluding extraneous eggs)	No. of composite genotypes	No. of secondary male alleles			Min. no. of secondary males	% secondary male progeny
				Str-A22/1	*Ssa*-A45/2	*Str*-A9		
1995								
A	4.9	78	48	6	5	4	3	55.1
B	0	49	30	5	4	3	3	34.7
C	0	52	26	0	0	0	0	0
D	2.8	103	69	8	5	4	4	48.5
E	0	117	37	2	2	2	1	2.6
F	0	88	46	4	6	6	3	51.1
G	28.9	68	41	8	7	4	4	30.4
1995 average (SD)	5.3 (10.6)	79.3					2.6 (1.5)	31.8 (22.6)
1996								
H	0	117	33	5	4	3	3	8.6
1995 and 1996 average (SD)	4.6 (10.0)	84					2.6 (1.4)	28.9 (22.5)

the use of an internal marker system, minimized error in typing alleles on autoradiographs (Fig. 1).

Combining data from all locations, 1726 individuals from 19 redds were screened at loci *Str*-A22/1, *Str*-A9, and *Ssa*-A45/2/1, detecting 13, 10, and 11 alleles, respectively. Between three and eight alleles were found per locus per redd, with average observed heterozygosities of 0.524–1.00 (Table 1). In most of the redd samples the majority of individuals had unique genotypes. In 18 of the 19 redds, additional alleles were present that could not have been contributed by a single male parent. Two redds (ranched A and wild H) were screened at two additional loci, but no further secondary male progeny were detected.

Subsampling

To estimate the effects of sample sizes on the estimates produced, 11 redds where sample numbers were greater than 50 were subsampled. Secondary male contribution was calculated from 50 randomly chosen individuals and compared with the original estimates (where the average sample size was 99.6). There was no significant difference between estimates (χ^2, $P = 0.625$), suggesting that a sample size of 50 is adequate to estimate secondary male contribution.

Wild redds

Eight redds known from observation to be formed by wild salmon were analysed (Table 2). Only one 1996 redd was analysed and data from both years were pooled for the purpose of analysis. Three redds contained extraneous embryos from another female(s) at an average frequency of 12.2%. These eggs were excluded from analysis, thus reducing the average sample size to 84 progeny per redd.

Seven of the eight redds analysed contained extrapaternal alleles in addition to the most common derived paternal genotype, i.e., the progeny of secondary males. Values of secondary male contribution ranged from 0 to 55.1%, with an average over both years of 28.9% (±22.5%). Up to eight extrapaternal alleles were found at a single locus. From this, the highest minimum number of secondary males per redd could be estimated as four (mean 2.6 ± 1.4).

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Table 3. Summary of genetic analysis of redds formed by ranched Atlantic salmon in the Burrishoole system.

Redd	% extraneous eggs	*N* (excluding extraneous eggs)	No. of composite genotypes	No. of secondary male alleles			Min. no. of secondary males	% secondary male progeny
				Str-A22/1	*Ssa*-A45/2	*Str*-A9		
1995								
A	26.7	99	37	4	6	4	3	61.6
B	16.7	60	41	5	4	3	3	65.0
C	29.6	143	65	5	6	5	3	70.7
D	60.5	19	13	4	2	3	2	42.1
E	24.7	67	54	7	5	4	4	65.7[a]
1995 average (SD)	31.7 (16.9)	77.6					3.0 (0.7)	61.0 (11.1)
1996								
F	0	117	37	5	4	5	3	25.0[b]
G	6.3	61	41	3	3	2	2	46.7
H	10.1	99	40	4	4	3	2	38.4[b]
I	25	45	35	4	4	5	3	53.3
J	19	47	32	3	5	4	3	27.7[c]
K	11.3	55	46	3	3	3	2	34.5[c]
1996 average (SD)	12.0 (8.9)	71.2					2.5 (0.5)	37.6 (10.9)
1995 and 1996 average (SD)	20.9 (16.1)	75.0					2.7 (0.6)	48.2 (16.1)

[a]Single hybrid progeny detected.
[b]Redds formed by genetically identical female and primary male.
[c]Redds formed by genetically identical female and primary male.

Ranched redds

Eleven redds, five sampled in 1995 and six in 1996, were analysed (Table 3). Data from 1995 and 1996 were analysed separately and together. Extraneous embryos were found in all but one of the redd samples at an average frequency of 20.9% over both years. In the most extreme case, in redd D, 29 of the 48 progeny sampled were from an extraneous source (based on mitochondrial DNA RFLP analysis as well as minisatellite variation). Over 2 years, the average sample size was reduced to 75 progeny per redd. A single salmon × brown trout hybrid was found among the progeny of the ranched redd E. Mitochondrial RFLP analysis confirmed that the embryo was unlikely to be from an extraneous source. Although the individual could be recognized as being hybrid due to minisatellite allele size differences at locus *Str*-A9, this was confirmed by analysis of 5S rDNA.

Secondary male progeny were detected in all of the ranched redds with contributions ranging between 25.0 and 70.7%, with an average value of 48.2% (±16.1%). The range of values found in 1995 samples was significantly higher than those in 1996 ($P = 0.006$), with four of the five 1995 values being higher than the 1996 range. The minimum numbers of secondary males did not vary significantly between years (Mann–Whitney *U*-test, $P = 0.329$). Overall, an average minimum of 2.7 (±0.6) secondary males were involved, with the highest number in redd E. No significant differences were found between the two ranched redd locations (Salmon Leap and Mill Race) with regard to proportion of secondary male progeny, numbers of secondary male alleles, or level of extraneous embryos.

From the putative parental genotypes, pairs of 1996 redds from the Salmon Leap site (F and H) and from the Mill Race site (J and K) appear to have been formed by the same females and primary males. If data from the paired redds are pooled, the average secondary male contribution among 1996 samples increases to 40.7%. However, this is still significantly different from the previous year's results ($P = 0.029$). The data for the pairs of spawning aggregations were not pooled for analysis because the interpretation of genotypic data may be incorrect, and because female spawnings may take place over a prolonged period of time (Fleming 1996), the two spawning aggregations may be considered as separate events. In the Mill Race pair of redds the majority of extrapaternal male alleles are shared, suggesting that the same secondary males were involved in both spawnings.

Comparison of ranched and wild redds

There were significantly more extraneous embryos detected in ranched than in wild redds ($P = 0.022$); however, sample sizes in the two groups were approximately equal. There was no difference in the minimum numbers of secondary males involved between the two groups (Mann–Whitney *U*-test, $P = 0.717$).

The proportions of secondary male contributions did, however, differ between groups ($P = 0.042$), with the highest four values coming from ranched redds. Wild redds had a wider distribution of values (0–55.1%) than ranched redds (25–70.7%), although the standard deviations of the averages were not significantly different (Bartlett's χ^2, $P = 0.639$). Overall, ranched primary males were found to achieve less than 75% of the success of wild primary males in fertilising ova. When results from 1995 only are considered, the average proportion of secondary male progeny among ranched redds (61.0%) was almost twice that of wild redds (31.8%), and comparisons were more significant ($P = 0.024$). Six of the 11 ranched redds were sampled in 1996, but only one of the wild redds; therefore, no comparisons can be made between 1996 samples alone.

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Discussion

The high levels of polymorphism of minisatellite and microsatellite markers and the ability to sample nondestructively have made it possible to unambiguously assign parentage and study reproductive success of individuals in natural populations of, for example, birds (Burke et al. 1991), poeclids (Schartl et al. 1993), and bluegill sunfish (*Lepomis macrochirus*) (Colbourne et al. 1996). The present study depended on the accurate reconstruction of parental genotypes from large samples of Atlantic salmon progeny and was carried out under natural conditions, unconstrained by the use of tanks or simulated stream environments.

Secondary male fertilisation of ova was widespread, occurring in 18 of the 19 redds examined. Of the 1484 progeny examined from Burrishoole, 40% could not be derived from the primary male genotype. Although these are likely to have been fertilised by mature parr, it is also possible that subdominant adult males may also have taken part in spawning (Fleming 1996). The methods used in this study do not enable subdominant male contributions to be distinguished from parr. However, in the absence of adult male biased sex ratios, the number of such spawning aggregations is likely to be small, and their presence does not affect the observation that secondary male contribution in ranched salmon redds is elevated. In many redds, no single locus detected all of the secondary male progeny. For example, in the ranched redd B, where 39 of the 60 progeny were contributed by secondary males, no more than 42% of these were detected by any one locus. Two redds were screened at two additional loci, but there was no evidence of other secondary male progeny, suggesting that in the absence of more variable markers, the three loci used produced satisfactory estimates.

Thomaz (1995) examined progeny from redds in the Aberdeenshire Girnock Burn, where ascending anadromous adults were sampled by trapping. Mature parr contributions averaged 36% (range 0–70%) and up to 11 extrapaternal alleles were found, indicating that at least six parr participated in some of the redds. Despite the differences in experimental design, similar proportions of secondary male progeny were detected in Burrishoole wild redds, suggesting that this is a generalized finding. Morán et al. (1996) used experimental channels to measure parr participation in three redds with varying numbers of parr (one, six, or 12) present. The highest level of parr contribution (90%) was found with six parr. In the absence of competition, a single parr was found to fertilise up to a quarter of the ova. Further, more detailed studies (Thomaz et al. 1997) were carried out in the same experimental channels to investigate the factors influencing parr success. Between 26 and 40% egg samples from five experimental redds were found to be fertilised by parr, with larger parr being dominant over smaller individuals, while increasing parr density was found to adversely affect relative parr success. However, the number of parr present in studies carried out under experimental conditions is predetermined and may not accurately reflect the situation in the wild.

Ranched redds

As previous work has linked increased levels of interspecific hybridisation to the occurrence of escaped farmed female salmon (Youngson et al. 1993), it is notable that the only hybrid progeny found in the study came from a ranched redd. Ranched redd E, in which the hybrid was found, had the second highest level of secondary male contribution and the largest number of secondary male alleles. This suggests that interspecific hybridisation may occur through the action of nonanadromous brown trout males fertilising Atlantic salmon ova. The apparent tolerance of ranched fish towards secondary males as seen in this study would provide more opportunity for hybridisation to occur.

A significant difference was found in the level of secondary male participation in ranched redds in the 1995 and 1996 samples. Unfortunately, while the data from ranched redds are spread evenly over two years, seven of the eight wild redd samples are from 1995. Only increased sampling of 1996 wild redds would indicate whether the decreased level of secondary male participation was an overall trend or was just among ranched redds. The variation between years could be due to the local environmental conditions adversely affecting the ability of parr in particular to participate in spawning. The dry autumn of 1995 prevented spawning adults ascending, and spawning in the Mill Race and Salmon Leap was delayed by 3–4 weeks. As well as being delayed, spawning was also concentrated in a short period of time. The late but rapid entry of the main body of spawners into freshwater may mean that too many adults were present at one time for parr to be as effective in spawning events compared with when spawning is spread over a longer period.

An unexpected finding was the widespread occurrence of embryos from two or more females in what was assumed to be a single redd. Such extraneous embryos were found in the majority of redds and at a significantly higher frequency in the ranched group ($P = 0.015$). There are three possible reasons for the occurrence of extraneous embryos.

(*i*) More than one female may have participated in the spawning event with a single male. However, there was no evidence in this study that extraneous embryos shared the paternal alleles found in the redd.

(*ii*) Fertilised ova may have drifted downstream from other redds.

(*iii*) Another female may have formed a redd over the top of the first, i.e., there may have been multiple use of the same site with the redd of interest overcutting ova already deposited in the gravel in spite of the redd being covered with wire mesh. Alternatively, a redd may have been already present in the gravel prior to the redd of interest. Overcutting may have elevated the incidence of extraneous embryos in the ranched redds, as fish were encouraged to spawn by the laying down of suitable substrate in defined areas.

In spite of the common occurrence of extraneous embryos, maternal genotypes could be determined with confidence. For example a female heterozygous at a locus would contribute each allele to progeny at approximately equal proportions. Maternal alleles could also be easily identified where progeny were homozygous. Mitochondrial DNA analysis, examining four polymorphic restriction sites, was also used to detect extraneous embryos where minisatellite variation proved ambiguous.

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Effects of fish culture on behaviour

This study has found a significantly greater level of secondary male contribution in the redds of ranched males and females than in wild redds. In the absence of other factors, the effect of culture on the ranched individuals must be considered as the likely cause. Cultivation, either by farming (where stocks are held in culture for multiple generations) or ranching, has been demonstrated to have a number of effects on Atlantic salmon, including phenotypic changes (Fleming and Jonsson 1994), change in run-timing (Jonsson et al. 1990), and reduction of genetic heterozygosity (Verspoor 1988). As discussed above, the increased level of interspecific hybridization (Youngson et al. 1993) indirectly suggests increased tolerance of cultured salmon towards secondary males. Jonsson et al. (1990) noted that a higher proportion of ranched (over one third of the males) than wild fish descended the Norwegian River Imsa without having spawned.

Anomalies in the spawning behaviour of cultured salmon may lead to an increased level of secondary male (particularly sexually mature parr) participation. In a comparison between farmed and wild salmon reproductive behaviour under experimental conditions, Fleming et al. (1996) found that farmed males tended to be less aggressive towards other males, and their spawning behaviour was often "inappropriate." Farmed females were on average a third as successful as wild females in terms of egg retention and egg survival in the redd. Studies of ranched males (Fleming et al. 1997) have produced similar results. Wild males were significantly more likely to be the only males involved in a redd whereas the redds of ranched males often involved two or more adult males (similar observations have been made in studies of ranched brown trout: Petersson and Järvi 1997.) However, it should be noted that no mature parr were included in the experimental design. The authors concluded that the lower level of reproductive success of the cultured males was due in part to the absence of typical juvenile learning behaviours. Wild parr experience territorial and competitive behaviour and, most importantly, may take part in spawning as mature parr.

Large numbers of farmed fish are known to escape from commercial operations and spawn in river systems (e.g., Webb et al. 1991; Crozier 1993; Lura et al. 1993). The results from this study suggest that due to the increased level of parr contribution, and consequent reduction of the paternal family size, the reproductive success of ranched female fish will be greater than that of ranched males. Therefore, there may be a higher level of gene flow from cultured to wild populations through the female lineage. Clifford et al. (1998*a*, 1998*b*) observed a disparity in the occurrence of mitochondrial and nuclear genetic markers that discriminated escaped farmed fish, with the level of the mitochondrial farmed genotype detected at a higher frequency. Such a situation could arise through relative differences in reproductive success between males and females. Having thus detected a difference in secondary male contribution due to changes in the reproductive behaviour of ranched fish, it would be appropriate to investigate similar effects in the redds of escaped farmed fish. By using "hybrid" spawning pairs of a farmed/ranched and a wild individual in experimental channels, the relative importance of males and females in limiting parr participation could be measured.

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Effect of animal and vegetal protein diets on feed intake and apparent digestibility of nutrients in rainbow trout (*Oncorhynchus mykiss*) infected by *Aeromonas salmonicida*, with and without chronic hypoxia stress

H. Néji and J. de la Noüe

Abstract: This study was undertaken to investigate the effects of bacterial infection (*Aeromonas salmonicida*) and chronic hypoxia on mortality, feed intake, and apparent digestibility of each of two diets in rainbow trout (*Oncorhynchus mykiss*). The diets were isonitrogenous (gross basis); one was based mainly on animal protein and the other on vegetal protein. In each of two separate and successive experiments, where the first served as a control for the second, fish were immersed on day 0 (d_0) in either a sterile phosphate buffer solution (first experiment) or a suspension of *A. salmonicida* (second experiment). Thereafter, the fish were either exposed on d_2 for 12 days to hypoxic conditions (60% of water oxygen saturation) or maintained under normal conditions. Feed intake was measured daily, while apparent digestibility coefficients for gross energy, dry matter, and crude protein were measured on d_0, d_2, d_5, d_9, d_{11}, and d_{14}. Among all infected fish, feed intakes were reduced ($P < 0.05$), regardless of the source of dietary protein or the degree of water oxygenation. Also, regardless of infection, the feed intakes of hypoxic fish that were fed the vegetal protein based diet were significantly less than those of fish that were fed the animal protein based diet. This response, however, was transitory, since normal levels of feed intake in the former fish were found by d_3. The apparent digestibility coefficients for all parameters were unaltered by hypoxia ($P > 0.05$), but they were significantly reduced ($P < 0.05$) by *A. salmonicida* infection, especially during the critical period of infection (d_5 and d_9).

Résumé : Le but de la présente étude est de déterminer les effets de l'infection expérimentale (par *Aeromonas salmonicida*) et d'un stress chronique d'hypoxie sur la mortalité, la prise alimentaire et la digestibilité apparente de l'énergie totale, de la matière sèche et des protéines brutes chez la truite arc-en-ciel (*Oncorhynchus mykiss*) nourrie avec l'un de deux régimes alimentaires isoprotéiques (base brute) et à base de protéine d'origine animale ou végétale. Durant chacune de deux expériences distinctes et successives où la première a servi de témoin pour la seconde, les poissons ont été immergés à jour 0 (d_0), soit dans une solution de tampon phosphate stérile (première expérience), soit dans une suspension bactérienne d'*A. salmonicida* (deuxième expérience) et soumis à d_2 à une réduction de l'oxygénation de l'eau (à 60% de la saturation de l'oxygène dans l'eau). Les mesures de la prise alimentaire ont été réalisées quotidiennement et celles du coefficient de l'utilisation digestive apparente des nutriments aux d_0, d_2, d_5, d_9, d_{11} et d_{14}. Les résultats montrent que la prise alimentaire est réduite ($P < 0,05$) par l'infection chez tous les poissons, indépendamment de la qualité du régime alimentaire et de la condition d'oxygénation, lors de l'hypoxie, et indépendamment de l'infection, la prise alimentaire est réduite ($P < 0,05$) chez les poissons nourris avec le régime végétal, alors que chez ceux nourris avec le régime animal, la réduction n'est qu'éphémère et un retour à la normale est constaté à partir de d_3 et le coefficient de digestibilité apparente des nutriments n'est pas affecté par l'hypoxie ($P > 0,05$), mais significativement ($P < 0,05$) réduit suite à l'infection par *A. salmonicida*, particulièrement durant la période critique qui correspond aux d_5 et d_9.

Introduction

Several studies have shown that mammals that were fed an animal protein diet were more resistant to stress than those that were fed vegetal or commercial diets (mixed protein sources) (Héroux and Roberge 1981; Thibault and Roberge 1988). Also, rainbow trout (*Oncorhynchus mykiss*) that were fed an animal protein based diet exhibited a greater resistance to hypoxic stress than those that were fed a vegetal protein based diet (Pouliot et al. 1987; Pouliot 1992). Other studies on fish have also shown correlations between immunity, resistance to disease, and nutritional status (Ghittino 1989; Landolt 1989; Blazer 1991). Moreover, Henken et al. (1987) noted that level of dietary intake affected antibody production in African catfish (*Clarias gariepinus*) following an injection of *Yersinia ruckeri*. Thomas

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Table 1. Constituents and proximate composition of experimental diets (% dry weight basis).

	Diet	
	Animal	Vegetal
Wheat middlings	34.5	—
Corn gluten meal	—	18.0
Poultry by-product meal	28.0	—
Feather meal	12.0	—
Soybean meal	—	65.0
Blood meal	11.5	—
Herring oil	10.2	11.1
Calcium phosphate	1.5	3.2
Sodium chloride	0.3	0.3
Endox 50	0.025	0.025
Choline	0.630	0.868
Vitamin premix[a]	0.2	0.2
Mineral premix[b]	0.25	0.25
Calcium carbonate	—	0.7
Lysine	0.64	0.3
Methionine	0.33	0.24
Total	100.08	100.2
Moisture[c]	7.07±0.13	7.39±0.18
Crude protein[c]	41.58±0.34	43.15±0.58
Lipid[c]	17.83±0.70	12.64±0.46
Ash[c]	5.65±0.45	6.48±0.57
Total energy (kJ/g)[c]	21.72±0.52	20.23±0.50

[a]Composition (per 1 kg of diet): vitamin A, 3332 IU; vitamin D_3, 2082 IU; vitamin E, 75 IU; vitamin K, 17.7 IU; vitamin B_{12}, 0.25 mg; riboflavin, 16.8 mg; pantothenic acid, 52.3 mg; niacin, 167 mg; folic acid, 8.3 mg; thiamine, 16.7 mg; pyridoxine, 12.5 mg; biotin, 0.165 mg; ascorbic acid, 333 mg.

[b]Composition (mg/kg diet): manganese, 43; zinc, 72; iodine, 4; iron, 31.5; copper, 12.4.

[c]Means ± SD (N = 4).

and Woo (1990) have also reported that a low protein diet modulated humoral immune responses and anemia in rainbow trout infected with *Cryptobia salmositica*.

The majority of infectious diseases appear shortly after exposure to environmental stress (Wedemeyer 1970; Anderson 1990). Despite the fact that infection is considered to be a nonspecific stress (Wedemeyer and Ross 1973), the relationship between diet, stress, and infection has yet to be investigated.

Some studies have described changes in performance and intestinal absorption in homeothermic animals suffering from pathogenic infection (Olsen and Richardson 1968; Reveron et al. 1974; Singh 1986). In fish, reduction in growth has been noted in trout feeding on commercial food contaminated with both *Aeromonas salmonicida* and other bacterial strains (Trust and Wood 1973). Previous research on nutrient digestibility in fish has been mostly limited to the effects of quantitative and qualitative changes in dietary composition (Windell et al. 1978; Cho et al. 1976, 1985; Hilton and Slinger 1986), pyloric caeca number (de la Noüe et al. 1989), water salinity (de Silva and Perera 1984), temperature (Choubert et al. 1982*a*; Fauconneau et al. 1983), and oxygen concentration (Bogé et al. 1979; Pouliot and de la Noüe 1988). The effect of infection on nutrient digestibility has not yet been investigated, except for a study by Ingham and Arme (1973). These researchers did not find any effect of *Eubothrium crassum* and *Proteocephalus* sp. cestode infection in rainbow trout on growth or intestinal absorption of L-leucine and D-glucose. Therefore, our goal in this study was to assess the singular interactive effects of hypoxia and *A. salmonicida* infection on mortality, feed intake, and apparent digestibility of dry matter, crude protein, and gross energy in rainbow trout that were fed either an animal protein based or plant protein based diet.

Materials and methods

Fish and experimental conditions

Two experiments were performed using a total of 360 rainbow trout. The fish originated from a certified disease-free farm and they were maintained at the experimental station of Université Laval in Saint-Augustin-de-Desmaures, Qué. (Canada). In each experiment, 180 fish were distributed randomly into twelve 60-L cylindrical tanks. Each tank contained 15 fish. The initial mean weights of fish (mean ± SD) were 150.7 ± 22.4 and 179.8 ± 35.5 g in experiments 1 and 2, respectively. The water in each tank was recirculated (closed-circuit system) after passage through a biological filter, and water flow rate and temperature were maintained at 4 L/min and 15°C, respectively. The other water quality parameters (NH_4^+, NO_2^-, and pH) were within the limits that have been recommended for rainbow trout culture (MAPAQ 1982). All fish were acclimated to the experimental conditions and to a 12 h light : 12 h dark photoperiod over a period of 20 days.

Experimental diets and feeding protocols

During the acclimatation and experimental periods, the fish in each group were fed one of two diets (Table 1) twice daily (09:00 and 16:00) at a ration level of 1.5% of their body weight. The diets contained similar levels of protein and gross energy (Table 1). However, they differed in protein sources, with one based mainly on animal sources and the other on a blend of soybean meal and corn gluten meal. Accordingly, the diets were designated as animal or vegetal (Table 1). Both diets were prepared in the laboratory. After weighing meals, oil and water were added to the ingredients. Subsequently, the mixture was put into a meat grinder equipped with a 4-mm-diameter die. Thereafter, the extruded product was dried, broken into pellets, and packed into black plastic bags. The bags were stored at –25°C until the experiments began. Chromic oxide (Cr_2O_3) was incorporated (at 1%) into each diet as an inert marker to determine the apparent digestibility coefficient (ADC, percent) according to Maynard and Loosli's (1969) formula. Feces were automatically collected (Choubert et al. 1982*b*) twice daily before the meals and then frozen, lyophilized, and kept at –40°C until analysis.

Experimental design

Two distinct and successive experiments were conducted. The first experiment served as a control for the second one. On day 0 (d_0) of each experiment, all fish in each of the 12 tanks were bathed in either a sterile buffer saline suspension (phosphate-buffered saline (PBS)) (experiment 1) or an *A. salmonicida* suspension (experiment 2). Fish immersed in the PBS suspension were termed "healthy fish" and those immersed in the *A. salmonicida* suspension were termed "infected fish." This technique of infection that we chose is described in Marquis and Lallier (1989) with minor modifications. In this regard, fish were removed from their tanks in groups of five, anesthetized with tricaine methanesulfonate (MS 222, 60 mg/L), weighed, marked, and immersed for 2 min in suspensions of *A. salmonicida* containing between 10^5 and 10^7 colony-forming units (cfu)/mL. After bathing, fish were

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rinsed in water and returned to their respective tanks. Five fish were bathed per litre of suspension.

After d_2 of each experiment, and for a period of 12 days, six groups of fish selected at random were maintained under normal conditions of water oxygenation ("normoxic fish") whereas the remaining six groups were exposed to hypoxic conditions ("hypoxic fish"). Water oxygen saturation for normoxic fish was maintained at 91.0 ± 4.5% (142 mm Hg (1 mm Hg = 133.322 Pa)) in experiment 1 and at 94.1 ± 4.0% (147 mm Hg) in experiment 2. In the case of hypoxic fish, water oxygen saturation was reduced to 56.9 ± 4.0% (88 mm Hg) and 54.7 ± 1.1% (85 mm Hg), respectively. Deoxygenation was achieved by using the system of Mount (1961, 1964). The oxygen concentration of water was measured with an oxygen meter (model 58, Yellow Springs Instruments Corp., Yellow Springs, Ohio).

In each of the two trials, three groups of normoxic and hypoxic fish were fed the animal protein based diet, while the remaining six groups (three normoxic, three hypoxic) were fed the vegetal protein based diet. Therefore, triplicate groups of fish were immersed in either PBS or *A. salmonicida* suspension, exposed to normoxia or hypoxia, and fed a diet based on animal or vegetal protein.

Each day, the dead and moribund fish were removed from the tanks before feeding and then identified. During the study, daily feed intakes were measured and feces were automatically collected according to the procedure mentioned above. We considered the feed intake to correspond to 100% when the fish ingested the whole ration distributed. The ADC of dry matter, crude protein, and gross energy was calculated on samples collected on d_0, d_2, d_5, d_9, d_{11}, and d_{14}.

Chemical analyses

Diets and feces were analyzed as follows: gross energy content was determined with an adiabatic calorimetric microbomb, dry matter content was measured by heating the samples at 94°C until constant weight was obtained, crude protein was measured with the automatic Kjelfoss method (percent nitrogen × 6.25), total lipid was determined with a Goldfish ether extraction apparatus, percentages of ash were determined after incineration of the samples at 550°C, and chromium levels were measured according to Arthur (1970).

Statistical analysis

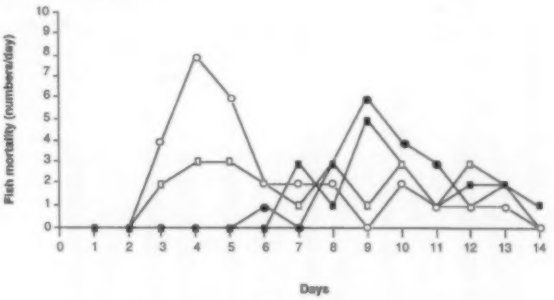
Analysis of variance (ANOVA) was performed for each diet level. A repeated measures ANOVA model (Montgomery 1984) was designed to ascertain the effects of infection, oxygenation, and day on the variation of ADC of dry matter, crude protein, and energy. The day was the repeated measure factor. Average tank values were considered units of observation for statistical tests. Differences between means were considered significant at $P < 0.05$. Duncan's (1971) multiple range test was used to identify significant differences between means.

Results

Mortality

None of the fish that were treated with sterile PBS in experiment 1 died. Also, no lesions were observed. However, the pattern of mortality for rainbow trout exposed to *A. salmonicida* was influenced by diet composition and water oxygen content (Fig. 1). The first deaths occurred on d_3 for the hypoxic fish and between d_6 and d_7 for the normoxic fish. In addition, regardless of diet, the highest daily mortality was recorded around d_4 and d_9 for the hypoxic and normoxic infected fish, respectively. Also, the daily mortality was often higher in the infected fish that were fed the vegetal protein based diet compared with the fish that were fed the animal protein based diet (Fig. 1). At the end of the experimental period, fish that had previously been fed the vegetal protein based diet accounted for 56.4% of the total number of dead fish.

Fig. 1. Daily mortality of rainbow trout infected with *A. salmonicida* on d_0 and exposed to hypoxia on d_2. Solid squares: animal protein based diet, normoxic fish; open squares: animal protein based diet, hypoxic fish; solid circles: vegetal protein based diet, normoxic fish; open circles: vegetal protein based diet, hypoxic fish.



Feed intake

Daily feed intake was reduced for all the *A. salmonicida* infected fish, regardless of dietary protein source (Fig. 2). With hypoxia, the reduction in feed intake was more severe for the fish that were fed the vegetal protein based diet compared with those that were fed the animal protein based diet, regardless of health status.

Perhaps because the immersion of fish in the PBS or the *A. salmonicida* suspension can be a stressful manipulation, all fish showed a marked reduction (about 15% from the normal level) in feed intake at d_0, regardless of dietary protein source or water oxygen content. The *A. salmonicida* infection led to a significant reduction in feed intake for the infected fish that were fed the animal protein based diet. This reduction began at d_5 in normoxia and at d_2 in hypoxia (Fig. 2*a*). After d_5, the level of feed intake became about 75% of the initial ration, regardless of the level of water oxygenation (Fig. 2*a*). However, healthy fish that were fed the animal protein based diet and subjected to hypoxia exhibited a transitory reduction in feed intake between d_0 and d_3 (Fig. 2*a*). Afterwards, feed intake remained around 100% (Fig. 2*a*).

In contrast, the foregoing changes were more severe for the fish that were fed the vegetal protein based diet (Fig. 2*b*). In this regard, feed intake decreased progressively from 40% (at d_3) to 15% of initial ration (at the end of experiments) for the infected fish exposed to hypoxia. Further feed consumption decreased from 85% (at d_2) to values varying between 55 and 70% of initial ration (at the second week of the experiments) for the infected fish exposed to normoxia (Fig. 2*b*). Feed intakes for healthy fish that were fed the vegetal protein based diet remained near 100% of initial ration in normoxia, from d_2 (Fig. 2*b*), but were reduced during hypoxia. In this situation, the values ranged from 64 to 93% (Fig. 2*b*). Chronic hypoxia therefore re-

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Fig. 2. Daily feed intake (means, $N = 3$) of rainbow trout fed the (*a*) animal protein based diet or (*b*) vegetal protein based diet, bathed in PBS or *A. salmonicida* suspension on d_0, and exposed to normoxia or hypoxia on d_2 (arrow). Solid circles: healthy normoxic fish; open circles: healthy hypoxic fish; solid squares: infected normoxic fish; open squares: infected hypoxic fish.

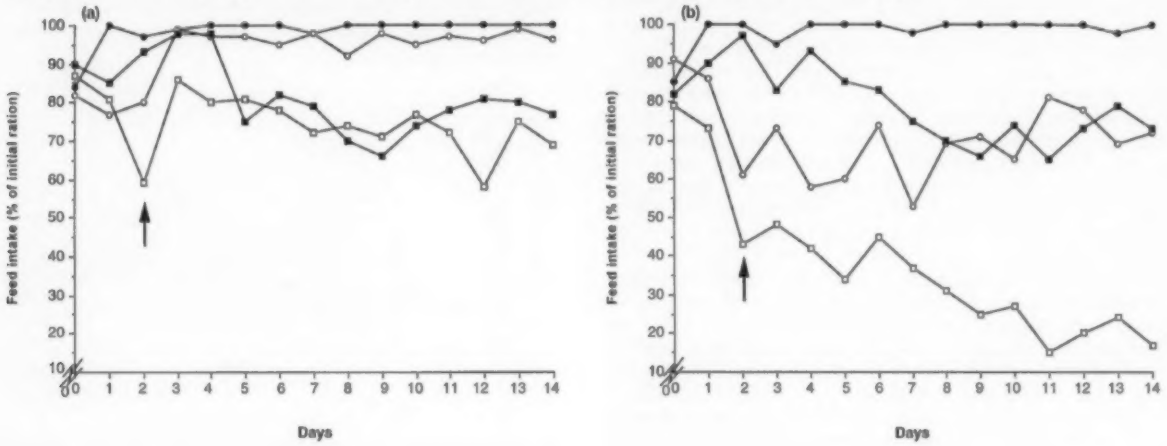

Table 2. ADC values (means ± SD, $N = 18$) for gross energy, dry matter, and crude protein for control and *A. salmonicida* infected rainbow trout subjected to either normoxia or hypoxia and fed and animal or vegetal protein based diet.

	Diet	Healthy normoxic fish (control)	Infected normoxic fish	Healthy hypoxic fish	Infected hypoxic fish
Gross energy	A	74.4±3.8	74.2±3.5	76.4±2.4	73.4±4.2
	V	78.9±3.3	75.4±3.5[a]	76.5±2.7	73.4±2.6[a]
	P^b	ns	ns	ns	ns
Dry matter	A	67.6±3.2	64.1±3.7[a]	69.4±4.3	64.3±4.0[a]
	V	69.7±2.7	67.1±3.2[a]	67.9±2.8	66.9±2.5[a]
	P^b	ns	ns	ns	ns
Crude protein	A	79.4±3.1	76.8±3.9[a]	81.0±3.4	75.5±4.8[a]
	V	93.1±4.2	87.3±6.3[a]	90.4±3.1	85.9±5.2[a]
	P^b	<0.001	<0.01	<0.001	<0.01

[a]Significantly different from control ($P < 0.05$).
[b]Effect of dietary protein sources: significant difference between fish fed the animal and vegetal protein based diets; ns, not significant ($P > 0.05$).

duced the feed intake of the healthy fish by more than 30% when they were fed the vegetal rather than the animal protein based diets. Following the *A. salmonicida* infection, reduction of feed intake was also more severe for the hypoxic fish that were fed the vegetal versus animal protein based diets. However, depression of feed intake was of similar magnitude for the infected normoxic fish, regardless of the source of dietary protein.

Digestibility

ADC values for crude protein were observed to be lower ($P < 0.05$) for the fish that were fed the animal protein based diet compared with those that were fed the vegetal protein based diet, regardless of fish health status and water oxygenation conditions (Table 2). *Aeromonas salmonicida* infection significantly depressed ($P < 0.05$) ADC values for dry matter and crude protein, regardless of nutritional status and water oxygen content. This result was also true for gross energy when the fish were fed the vegetal protein based diet but not the animal protein based diet (Table 2). However, 12 days of hypoxia did not lead to any significant changes ($P > 0.05$) in the ADC values for any of the parameters, regardless of the source of dietary protein.

Lower ($P < 0.05$) ADC values for dry matter, crude protein, and gross energy generally occurred on d_5 (Fig. 3) and d_9 (Figs. 3*a*, 3*b*, 3*c*, 3*e*, and 3*f*) for the *A. salmonicida* infected fish compared with the healthy fish, regardless of differences in water oxygen conditions and diet composition. In addition, lower ($P < 0.05$) ADC values for gross energy (Fig. 3*b*) and crude protein (Fig. 3*f*) were noted on day d_2 for the *A. salmonicida* infected fish compared with the healthy fish when these fish were exposed to hypoxia and fed the vegetal protein based diet.

Moreover, the factor day had no effect ($P > 0.05$) on ADC values for energy and nutrients in healthy fish, regardless of the experimental conditions (Fig. 3). However, significant reductions ($P < 0.05$) in ADC values for crude protein occurred on d_5 and d_9 for the infected hypoxic fish that were

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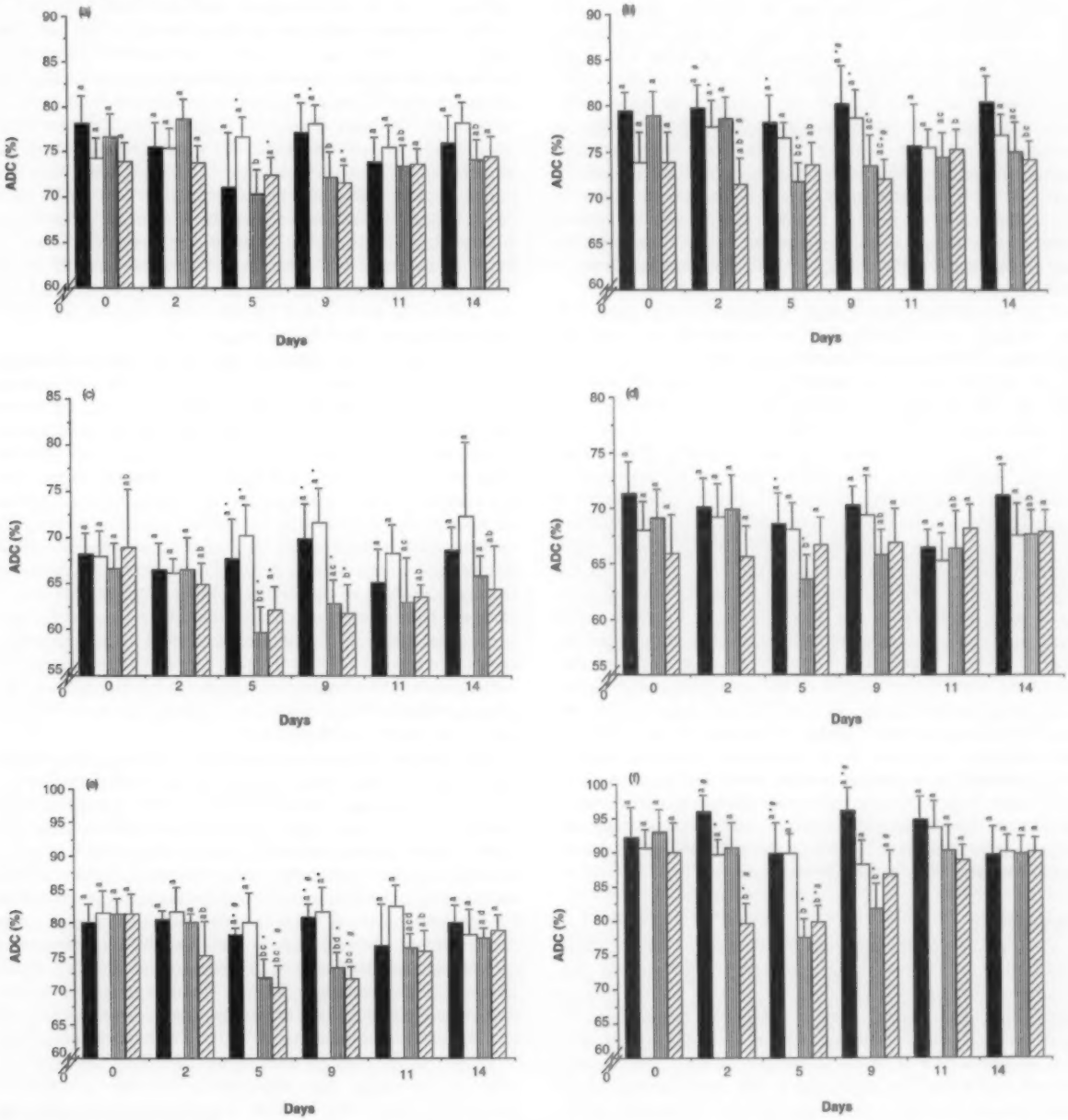
Fig. 3. ADC values (means ± SD, $N = 3$) for (*a* and *b*) gross energy, (*c* and *d*) dry matter, and (*e* and *f*) crude protein in rainbow trout fed the (*a*, *c*, and *e*) animal protein based diet or (*b*, *d*, and *f*) vegetal protein based diet, infected with *A. salmonicida* on d_0, and exposed to hypoxia on d_2. Solid bars: healthy normoxic fish; open bars: healthy hypoxic fish; shaded bars: infected normoxic fish; hatched bars: infected hypoxic fish. Means with different letters indicate, for each experimental treatment combination, a significant difference ($P < 0.05$) between the days. An asterisk indicates, for the same day and oxygen conditions, a significant difference ($P < 0.05$) between healthy and infected fish. A pound sign indicates, for the same day, a significant difference between healthy normoxic and infected hypoxic fish.



fed either the animal or the vegetal protein based diets (Figs. 3*e* and 3*f*). Reductions in ADC values for energy on d_2 and d_9 (Fig. 3*b*) and for crude protein on d_2 (Fig. 3*f*) were recorded only for the infected hypoxic fish that were fed the vegetal protein based diet.

Discussion

Feed intake

The slight decreases in feed intake that were observed at the beginning of the experimental period (d_0) and on the day

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of initiation of hypoxia (d_2) could be attributed to the primary or secondary effects of stress (Mazeaud et al. 1977). These would have resulted from the numerous manipulations that were done to carry out the arduous infection protocol (for day d_0) and from exposure of rainbow trout to hypoxic conditions on d_2 (Fry 1969).

Several authors have shown depressions of feed intake similar to the ones obtained here for hypoxic rainbow trout as a consequence of reducing water oxygen concentration from 12 to 4 mg O_2/L (Pederson 1987) or from 9 to 5 mg O_2/L (Medale et al. 1987). The return of feed intake to prestress levels occurred 2 days after initiation of hypoxia for fish that were fed the animal protein based diet. This response did not occur in those fish that were fed the vegetal protein based diet. It is possible that the dissimilar feed intakes of the fish in this study, due to differences between diets in the source of protein, can be explained by the animal protein improving the resistance of the fish to stress (Héroux and Roberge 1981; Pouliot et al. 1987; Thibault and Roberge 1988; Pouliot 1992).

The *A. salmonicida* infection reduced the feed intakes of the rainbow trout. The depressions of feed intake, often the first sign of fish disease under natural conditions, were influenced by the source of dietary protein, especially for the hypoxic fish. Compared with the infected fish that were fed the animal protein based diet, the fish that were fed the vegetal protein based diet showed greater decreases in their feed intake. This may have been due to the strongly documented relationship between feed intake and severity of infection in animals. The lowest feed intakes have often been observed in the most infected animals (Beisel 1984; Symons 1985; Henken et al. 1987; Thomas and Woo 1990, 1992). On the basis of feeding behaviour, we suspect that the rainbow trout were more sensitive to the *A. salmonicida* infection when they were fed the vegetal protein based diet rather than the animal protein based diet. Thus, the pathogenicity of furunculosis could be influenced by the source of protein in the diet, as demonstrated by Wedemeyer and Ross (1973) for bacterial kidney disease (BKD). Alternatively, the differential response may have been due to limitations of one or more nutrients as a consequence of altering the source of dietary protein (e.g., one or more minerals) or to the presence of one or more deleterious components accompanying the vegetal protein sources. Our data do not allow us to distinguish between these possibilities.

Digestibility

It is evident that the apparent digestibility of crude protein was higher for rainbow trout that were fed the vegetal protein based diet than for fish that were fed the animal protein based diet. The vegetal protein based diet contained 65% soybean meal, and it is known (Cho et al. 1985) that the protein in this source is highly digestible in trout. The animal protein based diet that was used in this study contained ingredients such as wheat middlings, poultry by-product meal, feather poultry meal, and blood meal that are known to have lower protein digestibility coefficients in trout (Cho et al. 1985) and to be highly variable among suppliers (Dong et al. 1993; Hajen et al. 1993). For instance, when feather meal was used as the sole animal protein source in a diet for rainbow trout, the digestibilities of protein, fat, and energy were found to be 62, 68, and 74%, respectively (Cho and Slinger 1979). On the other hand, most experiments on rainbow trout have shown good growth and feed conversion ratios when the diet contained poultry by-product meal, feather meal, methionine, and lysine (Alexis et al. 1985; Steffens 1987). According to Steffens (1994), the waste material of the poultry industry may be recommended as a suitable and fish meal sparing ingredient for rainbow trout.

The reduction in dissolved oxygen content in the water (hypoxia at 60%) did not have a measurable effect ($P > 0.05$) on the ADC values of dry matter, gross energy, and crude protein. These results are in agreement with those of Pederson (1987) and Pouliot and de la Noüe (1988) who directly and indirectly measured the digestibility of nutrients, respectively. These authors exposed their hypoxic fish to a water oxygen saturation corresponding to 4–6 mg O_2/L in the former case (Pederson 1987) and 40% in the latter (Pouliot and de la Noüe 1988). Thus, it seems that the threshold level of hypoxia that leads to a significant change in the ADC values of nutrients must be lower than the level that we used in the present experiment or that hypoxia has no effect on nutrient digestibility.

The reductions in nutrient and energy digestibility that were noted in rainbow trout infected with *A. salmonicida* ($P < 0.05$) may have been due to the pathogenic effects of this bacterium. Despite the presence of petechiae and hemorrhagic lesions that were observed in the intestines of some dead and moribund fish, the anatomical damage alone could not explain the impairment of digestibility. Indeed, *A. salmonicida* does not possess selective affinity in the digestive tract after a bath challenge model (Smith 1982; Tatner et al. 1984; Rose et al. 1988). We therefore think that the small quantity of bacteria remaining in the digestive tract after the bath was insufficient to significantly alter the anatomical integrity of the digestive tract. Using autoradiography of a labeled soluble antigen, Smith (1982) showed that small amounts of antigen were taken up by the skin and gut after a bath challenge, while the majority of the radioactive label was found in the gills.

It is possible that reduced nutrient and energy digestibility in diseased rainbow trout was caused by stimulation of the sympathetic nervous system, which controls gastrointestinal motility in mammals (Ito and Kuriyama 1971; Campbell 1975). Consequently, reduced nutrient absorption can occur because of activation of the sympathic nervous system. Possibly, such activation occurs during the critical period of furunculosis infection when a high level of bacterial toxins is released into several body tissues. Therefore, among other effects induced, the bacterial toxins may have led to a stimulation of digestive tract motility. This explanation is supported by the finding that the highest daily mortality (Fig. 1) and the greatest reductions ($P < 0.05$) in nutrient digestibility (Figs. 3*b*, 3*e*, and 3*f*) were observed during the same period. Accordingly, the stimulation of the sympathetic nervous system in the infected fish may have occurred between d_5 (Figs. 3*e* and 3*f*) and d_9 (Figs. 3*b*, 3*e*, and 3*f*).

Simultaneous exposure of rainbow trout to *A. salmonicida* infection and chronic hypoxia stress irrespective of diet treatment led to significant reductions ($P < 0.05$) in the ADC values of nutrients on d_2 (Figs. 3*b* and 3*f*), d_5, and d_9 compared with healthy normoxic fish. Since it was too early for

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the *A. salmonicida* to destroy a large surface of the digestive tract, the depressions noted on d_2 for the ADC values of nutrients in the infected fish do not seem to have the same origin as those observed on d_5 and d_9. Thus, the reductions in ADC values of nutrients on d_2 could only be attributed to a strong perturbation in the homeostasis of the fish. This possibly may have been the result of additive primary and secondary effects of the two stressful events (Floss et al. 1988), viz. the initiation of hypoxia and the nonspecific stress caused by infection.

Possible relationships between the infective concentrations of *A. salmonicida* (10^5, 10^6, and 10^7 cfu/mL) and the ADC values of nutrients could not be measured directly in our experiments because all the infected fish were mixed together in the same tank. Thus, the feces were pooled before their collection. Nevertheless, a relationship between the severity of infection and the level of nutrient digestibility has been well demonstrated in several terrestrial species, such as swine (Stewart et al. 1983), poultry (Takhar and Farrell 1979), lambs (Reveron et al. 1974), and calves (Cole et al. 1986).

Overall, our findings suggest that feed intake and apparent digestibility of nutrients can be adversely affected in rainbow trout when negative environmental factors alter the physiological state of the fish. Some of these factors, such as the level of hypoxia used in the present experiment, do not change the digestibility of nutrients despite reductions in feed intake. Further, the exposure of rainbow trout to a virulent strain of *A. salmonicida* generally led to depression of dry matter, protein, and energy availability, regardless of the source of dietary protein. Depressed nutrient digestibility, especially during the critical period of infection (d_5 and d_9), likely deprives the fish of metabolic fuel at the moment when it is most needed. Thus, this research confirms for the first time that a quantitative reduction of macronutrient absorption occurs during infection. Hence, the application of any nutritional strategy aimed at increasing the resistance of rainbow trout and perhaps other salmonids to stress could be compromised during the period of infection. More work, especially with regard to postabsorption events, is necessary to explain why the infected fish that were fed the vegetal protein based diet showed a higher mortality compared with fish that were fed the animal protein based diet, especially when the latter had lower available protein content. Also, further studies are needed to understand what happens to the development of resistance to stress in rainbow trout after using a commercial diet based often on fish meal. Again, it is important to evaluate the involvement of soybean meal, which is a major ingredient in the vegetal protein diet and contains some antinutritional factors, such as phytic acid, that are responsible for impairment of nutrient bioavailability.

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Respiratory metabolism and swimming performance in growth hormone transgenic Atlantic salmon

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Abstract: We show that growth hormone enhanced transgenic salmon have a higher oxygen uptake during routine culture conditions and during forced swimming activity relative to similarly sized control fish. They also have a slightly higher critical oxygen level that limits oxygen uptake. However, they do not differ in regards to critical swimming speed. Growth hormone transgenic Atlantic salmon, *Salmo salar*, at 12–13°C were an F_2 generation using eggs from a transgenic F_1 female and milt from a nontransgenic male. They grew two to three times faster than control fish throughout the study period. Under routine culture conditions, both transgenic and control fish exhibited a diurnal cycle in oxygen uptake. However, oxygen uptake of transgenic fish was 1.7 times that of control fish at all times of the day. Oxygen uptake was independent of oxygen concentration above 10 mg/L in both groups of fish; critical oxygen uptake level was 6 mg/L in transgenic fish and 4 mg/L in control fish. During the oxygen decrease, transgenic fish and control fish lost equilibrium at the same low oxygen concentration levels. In the swim tunnel, oxygen uptake of transgenic fish was 1.6 times that of control fish at all swimming speeds. Critical swimming speeds did not differ between transgenic and control fish and were similar to literature values for salmonids.

Résumé : Nous montrons que les saumons transgéniques améliorés pour l'hormone de croissance présentent une consommation d'oxygène plus élevée dans les conditions d'élevage habituelles et pendant des exercices de nage forcée, comparativement à des poissons témoins de taille semblable. Ces saumons présentent aussi une teneur d'oxygène critique légèrement supérieure qui limite la consommation de l'oxygène. Ils ne présentent cependant pas de différence en ce qui a trait à la vitesse de nage critique. Les saumons transgéniques pour l'hormone de croissance étaient des saumons de l'Atlantique, *Salmo salar*, maintenus entre 12 et 13°C qui avaient été obtenus (F_2) à partir d'oeufs d'une femelle transgénique (F_1) et de laitance d'un mâle non transgénique. Pendant la période d'étude, leur croissance a été de deux à trois fois supérieure à celle des témoins. Tant les poissons transgéniques que témoins ont présenté un cycle diurne de consommation de l'oxygène en conditions d'élevage normales, mais la consommation d'oxygène des poissons transgéniques correspondait à 1,7 fois celle des poissons témoins, cela pendant toute la journée. Chez les deux groupes de poissons, la consommation d'oxygène était indépendante de la concentration d'oxygène au delà de 10 mg/L mais le niveau critique de consommation d'oxygène était de 6 mg/L pour les poissons transgéniques et de 4 mg/L pour les témoins. Pendant l'étape d'appauvrissement en oxygène, les poissons transgéniques et les témoins ont perdu l'équilibre aux mêmes faibles concentrations d'oxygène. Dans le tunnel de nage, la consommation d'oxygène des poissons transgéniques était de 1,6 fois supérieure à celle des témoins, cela à toutes les vitesses de nage. Les vitesses de nage critiques étaient les mêmes chez les poissons transgéniques et les témoins et semblables aux valeurs signalées dans les publications traitant des salmonidés.

[Traduit par la Rédaction]

Introduction

Farmed fish account for a large and ever-increasing fraction of Atlantic salmon, *Salmo salar*, that appear on the market. Considerable effort has been put into decreasing the time from spawning to achieving marketable size. Optimization of abiotic factors such as temperature, photoperiod, and salinity have produced significant results (Duston and Saunders 1995). In addition, manipulation of biotic factors such as selective breeding, ration size, food quality, and injection of hormones (especially growth hormones) also have produced significant results. More recently, dramatic improvements in growth rate have been achieved using techniques in molecular genetics. In particular, transgenic fish have been developed in which genetic material from one or more species has been transferred to another. Salmon growth hormone (GH) attached either to antifreeze protein promoter (Du et al. 1992) or to metalothionein-B promoter (Devlin et al. 1994) both have been used successfully to realize large increases in salmonid growth rate.

Rapid growth might come with some metabolic cost. We had three reasons to expect an elevated oxygen uptake in transgenic fish. First, in the course of developing the use of growth-enhanced transgenic salmon at AquaBounty Farms, Prince Edward Island, Canada, it has been routinely ob-

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Table 1. History of the fish used in the present study. Development of the transgenic fish was delayed by keeping the eyed eggs at a lower temperature.

		Control			Transgenic		
Date		Weight (g)	Temperature (°C)	Days fed	Weight (g)	Temperature (°C)	Days fed
1 Nov. 1996	Spawning						
	Egg incubation		7			4	
19 Feb. 1997		0.12	13	1		5	
8 Mar. 1997		nd	13	18	0.12[a]	13	1
28 Apr. 1997		0.78	14	69	0.50[a]	14	52
26 May 1997		2.04	15	97	1.56[a]	15	80
12 June 1997		3.27	15	114	3.06[a]	15	97
25 June 1997	Graded	nd	14	127	5.84[b]	14	110
8 July 1997		nd	16	140	9.36[b]	16	123
20 July 1997		6.98	14	152	13.72[b]	14	135
3 Aug. 1997		9.28	14	166	24.93[b]	14	149
17 Aug. 1997		11.06	14	180	38.92[b]	14	163
31 Aug. 1997		13.67	14	194	55.76	14	194
14 Sept. 1997		16.69	13.5	208			
29 Sept. 1997		23.45	13.5	223			
12 Oct. 1997		28.09	13.5	236			
26 Oct. 1997		32.07	13.5	250			
9 Nov. 1997		37.22	13.5	264			
22 Nov. 1997		43.35	13.5	277			
14 Dec. 1997		52.91	13.5	299			

Note: The intent was to have both groups with a wet body weight about 10 g in mid-July. nd, no data (weight was not measured on that day).

[a]Total population (i.e., both UMG and LMG).

[b]UMG of the transgenic fish. Control fish were also graded and larger individuals were used; however, the frequency distribution was unimodal rather than bimodal.

served that transgenic fish have a higher metabolic rate than the same biomass of nonenhanced fish as evidenced by lower oxygen levels in rearing tanks. Second, Hogendoorn (1983) showed that there is a direct linear relation between the amount of oxygen consumed (not the rate of consumption) and the dry weight gain for some fish when on different ration levels. Third, there is also reason to believe that GH may increase metabolic rate because injections of exogenous ovine GH in fish can elevate oxygen uptake (Seddiki et al. 1996).

The purpose of the present study was to quantify the oxygen uptake rate of transgenic fish relative to that of control fish under different conditions. First, we measured oxygen uptake during routine culture conditions. Second, we measured oxygen uptake in relation to ambient oxygen concentration addition. Third, because activity is a major metabolic cost, we measured oxygen uptake when the fish were forced to swim at various water velocities in a swim tunnel. This also allowed us to measure maximum swimming performance (critical swim speed or U_{crit}).

Methods

Experimental fish

Both the production of and research on the transgenic fish used in these studies took place at the AquaBounty hatchery in Prince Edward Island, Canada. This facility is government inspected with respect to the required containment measures for working with genetically modified fish. All fish used in all of the crosses and all control fish are from St. John River stock Atlantic salmon. The gene construct is comprised of an antifreeze protein gene promoter from the ocean pout, *Macrozoarces americanus*, and the growth hormone gene from chinook salmon, *Oncorhynchus tshawytscha* (Hew et al. 1995). Approximately 10^6 copies of the gene construct were injected through the micropyle of normal fertilized but nonactivated eggs in 1989 according to the method of Shears et al. (1992). One of the fast-growing males (P_1 or F_0) from this lot of eggs was crossed with a nontransgenic female in fall 1991. A fast-growing female that resulted from this cross (F_1) was crossed with a nontransgenic male in fall 1994 (F_2) and again in fall 1996 (another F_2). In both 1994 and 1996, the fish developing from these crosses had a clear bimodal size distribution. The two modes could be separated with about 90% certainty at 5 g mean body weight and with 100% certainty at 10 g mean body weight (confirmed using polymerase chain reaction). The proportion of fish in the UMG (upper modal group) to those in the LMG (lower modal group) was 50:50 in both spawnings, which is consistent with a gene insert on a single chromosome. The transgenic fish used in the present study were the UMG from the 1996 spawning.

A major difficulty in these types of studies is finding an adequate control to compare with the transgenic fish. For most of the present study we used offspring from a nontransgenic cross that was spawned at the same time and from the same stock (Table 1). To have fish approximately the same size (about 10 g) at the same time (mid-July), the transgenic eyed eggs were initially incubated at a lower temperature (4°C) relative to control fish (7°C) to slow their embryonic development (Table 1). As a consequence, time at first feeding was delayed about 17 days in the transgenic fish. All fish were reared in 92-L flow-through tanks at 12–13°C and fed to satiation three times daily with standard commercial pellets (Shur-Gain optima salmon ration). Water used for rearing and for all of the experimental trials had the following properties: hardness as $CaCO_3$ was 150 mg/L, pH was 7.6, and salinity was 4‰.

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Table 2. Physical characteristics and critical swim speeds of fish used in the present study.

(A) Fish used in the routine oxygen uptake experiments.

	Control fish (n = 50)	Transgenic fish (n = 53)	P
Fork length (cm)	13.60±0.10	13.56±0.59	ns
Wet weight (g)	28.95±0.52	22.99±0.11	0.001
Condition factor	1.15±0.003	0.91±0.004	0.001
Growth rate (%/day)	1.29	3.37	

(B) Fish used in oxygen dependence of oxygen uptake rate experiments.

	Control fish (n = 40)	Transgenic fish (n = 39)	P
Fork length (cm)	15.04±0.15	17.78±0.19	0.001
Wet weight (g)	40.76±1.12	49.81±1.41	0.004
Condition factor	1.19±0.02	0.88±0.01	0.001
Growth rate (%/day)	1.04	2.75	

(C) Fish used in swimming speed tunnel experiments.

	Control fish (n = 16)	Transgenic fish (n = 15)	P
Fork length (cm)	9.30±0.13	10.79±0.17	0.001
Wet weight (g)	8.42±0.27	10.53±0.57	0.004
Condition factor	1.04±0.02	0.83±0.01	0.001
U_{crit} (cm/s)	44.8±1.40	44.2±2.65	0.83
U_{crit} (BL/s)	4.83±0.16	4.11±0.26	0.09
Growth rate (%/day)	1.72	3.67	

In all experiments, an Oxyguard handy mark 4 oxygen sensor/monitor equipped with analogue output jacks (Point Four Systems Inc., Port Moody, B.C.) and a chart recorder were used to measure oxygen levels. The OxyGuard probe is a galvanic cell; it is not a Clark-type electrode. This self-polarizing probe produces a millivolt signal directly proportional to the oxygen content. The measured time constant of the sensor was 73 s; it was calibrated daily using water bubbled with air and zeroed using water bubbled with nitrogen.

Routine oxygen uptake

As part of an ongoing experiment to compare long-term feed utilization, provisions were made to seal the 24 rearing tanks (92 L each) using Plexiglas covers that clamped down over rubber gaskets. The fish had been reared in these tanks for at least 1 month prior to the current experiments and were accustomed to being fed three times a day through a feeding tube in the cover that prevented water loss or the introduction of air. This respirometer tank contained a small submersible pump to maintain a slow current velocity. Heated well water entering these tanks was first stripped of excess nitrogen using oxygen, followed by air stripping to remove excess oxygen and finally cooled 0.5°C (from 13 to 12.5°C) to prevent bubble formation on the tank surfaces. The tanks were closed for the periods of time necessary for the oxygen to drop from near air saturation (10 mg/L) to no lower than 6 mg/L, then tanks were flushed to restore oxygen to original levels. This process was continued for a period of 33–39 h. To control for biofilm metabolism on the tank surfaces, fish were removed from the tank the following day and a tank constant was measured to correct for uptake other than that by the fish. Oxygen uptake was measured for 53 transgenic fish in late July; identical methods and conditions (also at 12.5°C) were used to measure uptake of 50 control fish when these fish had reached comparable size in mid-October.

Oxygen dependancy of oxygen uptake

Using the same tanks, similar methods were used to estimate the critical oxygen concentration (level at which oxygen uptake became limited by oxygen supply), except the initial oxygen concentration was increased to about 20 mg/L and allowed to decrease to levels at which the fish lost equilibrium (about 2 mg/L). A tank containing 40 fish was sealed, but water flow was maintained, and the fish were fed through the feeding tube as usual. On the test day, water inflow was stopped for the measurement trial and oxygen concentration was monitored as it was consumed. A TV camera was mounted above the tank to measure the time at which fish lost equilibrium. Carbon dioxide accumulates as the oxygen is consumed in this type of setup, but because of the hardness of the well water, pH changes were not significant. These experiments were done after the swim tunnel experiments when the transgenic fish had grown to about 50 g. Hence, a different control was employed consisting of nontransgenic fish that were 1 year older, which had been lower mode parr the previous fall but had not reached sufficient size to become 1-year smolts in the spring of 1996. Throughout the winter and spring these control fish were held at 7°C and gradually acclimated to 13°C, 1 month prior to the experiments. During the time of the experiment (August 1997) the control fish (1+ parr) were of comparable size to the 0+ transgenic fish. That is, the control fish were exactly 1 year older than the transgenic fish but similar in size (Table 2). Water temperature was 12.5°C during the trials.

Swimming respirometry

The swim tunnel respirometer was a 3.6-L Blazka type with an inside diameter of the inner tube of 5.8 cm. It was the last one built by the late Fred Fry and was the same one used by the late George Holeton in most of his studies (Fry 1971, Fig. 3A). Nominal speeds used were 20, 30, 40, 50, and 60 cm/s. Fish were trained in groups of three in a similar tunnel for 10 h at about 20 cm/s prior to being tested and were fed on the day prior to the test but not fed on the test day. Mean water temperature was 12.5°C and was controlled by running water over the respirometer; ambient air temperature in the hatchery varied from 12 to 13°C during the course of this study. A small voltage (12 V AC) was applied to the grids at the back of the tunnel only when the fish touched the back grid. Optomotor cues consisted of vertical black lines, 1 cm wide, painted on the front half of the outside of the outer chamber.

To measure oxygen uptake, the respirometer was sealed and the slope of the decrease in oxygen concentration was estimated from pen recorder tracings from an oxygen sensor in the outer chamber of the respirometer. Blanks were run every day and uptake rates were subtracted from fish rates; blank rates always were less than 5% of fish rates. The respirometer was disinfected every day with ovadine (iodophore solution commonly used in hatcheries). The cross-sectional area of the largest fish tested in the respirometer was less than 8% of the cross-sectional area of the tube so no correction for solid blocking was applied. Cross-sectional area of the largest fish was estimated by cutting a cross-section of the fish and measuring its area.

During a swim test a fish was left at a speed of 20 cm/s for 1 h, and then speed was increased every 30 min in 10-cm/s increments. The speed was increased slowly over a 1-min period. Oxygen uptake was estimated from the last 20 min of the 30-min period at each new speed. Oxygen concentration never went below 10 mg/L. Critical swimming speed was calculated using the method of Bell and Terhune (1970) from the duration at the fastest speed and the speed increment.

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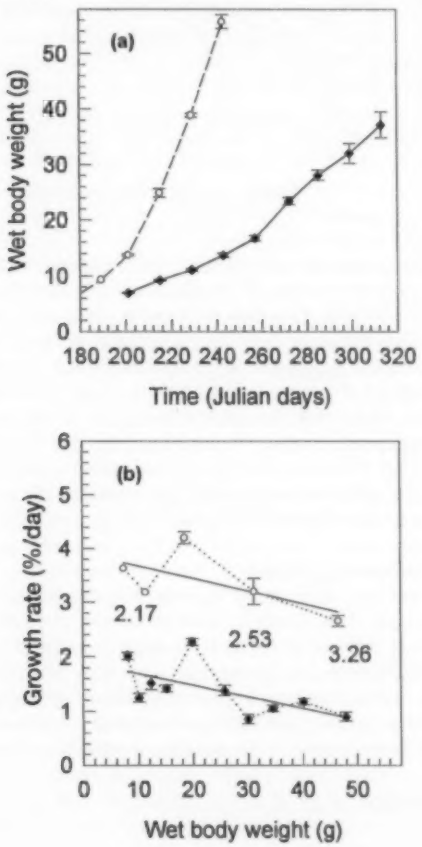
Fig. 1. (*a*). Growth as reflected by the change in wet body weight with time of the transgenic fish (open circles, broken line) used in all experiments and the control fish (solid diamonds, solid line) used in the routine oxygen uptake and swim tunnel experiments. Fish were spawned on the same day, but development of the transgenic fish was delayed by rearing the eggs at a lower temperature for 17 days. Values are means from six rearing tanks each containing at least 50 fish. Error bars are SE. (*b*). Weight-specific growth rate as a function of mean body weight over the interval. Solid lines are least-squares regression lines. The weight-specific growth rate of the transgenic fish calculated from these regression lines was 2.17 times that of control fish at 10 g, 2.53 times at 30 g, and 3.26 times at 50 g.

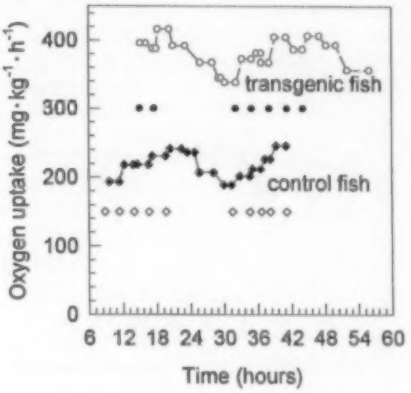


Results

Growth

The growth data in Fig. 1*a* shows the mean fish weight of at least six replicate tanks, each containing at least 50 fish from each of the two groups of fish fed excess ration and at a temperature of 12–13°C. Weight-specific growth rate (Ricker 1979) was calculated for each interval (Fig. 1*b*) and tended to decrease in both groups of fish over the course of the experiment. The instantaneous growth rate of the transgenic fish was 2.2 times that of the control fish when the fish were 10 g body weight and was 3.3 times that of the control fish when the fish were 50 g body weight.

Fig. 2. Routine oxygen uptake of transgenic fish (open circles, broken line) and the control fish (filled diamonds, solid line) as they consumed the oxygen in the tank respirometer over the period of about 1.5 days. Feeding times are the points below each oxygen uptake line (solid circles, transgenic fish; open diamonds, control fish).



Transgenic fish were slightly longer, slightly heavier, and had a lower condition factor than control fish (Table 2). They tended to become more silvery earlier (midsummer) and at a smaller size than control fish did. Their fins had darker margins and parr marks were less obvious, whereas control fish did not show these changes in silvering until later in the season, which is typical for nontransgenic fall presmolts cultured in hatcheries. All of the fish used in these trials, transgenic and control fish, always orientated and swam against the current in their rearing tanks; none ever swam with the current.

Routine oxygen uptake

Both transgenic and control fish of comparable body size exhibited 24-h cycles in oxygen uptake with rates lowest at dawn and increasing throughout the day when they were fed to a maximum in late evening (Fig. 2). Integrating these curves over 24 h yielded an oxygen uptake of 375 $mg \cdot kg^{-1} \cdot h^{-1}$ for 23-g transgenic fish compared with 220 $mg \cdot kg^{-1} \cdot h^{-1}$ for 29-g control fish, both at 12.5°C. Therefore, when fed excess ration five times a day, these transgenic fish had a routine oxygen uptake 1.7 times that of control fish.

Oxygen dependancy of oxygen uptake

Oxygen uptake was independent of oxygen concentration above 10 mg/L (153 torr) but increased slightly because of increased activity and then became depressed at lower oxygen levels (Fig. 3*a*). The critical oxygen level, that is, the oxygen level at which the oxygen uptake rate started to decrease, was slightly higher in transgenic fish. It was about 6 mg/L in transgenic fish and about 4 mg/L in control fish (Fig. 3*a*). Oxygen uptake had decreased 50% when the oxygen concentration had decreased to about 2.9 mg/L in transgenic fish and to about 2.7 mg/L in control fish. Control fish and transgenic fish lost equilibrium at the same oxygen level (Fig. 3*b*). The first fish started to lose equilibrium when the

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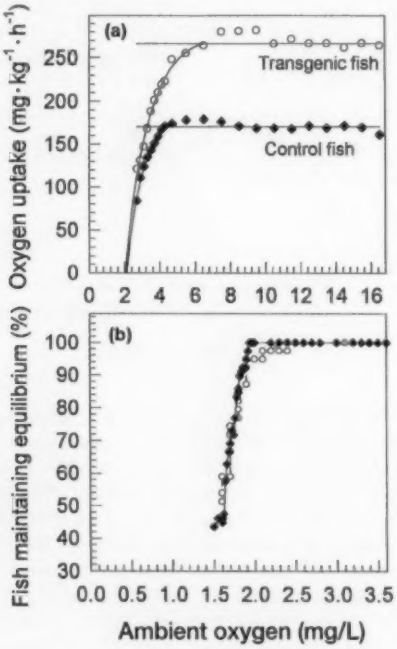
Fig. 3. (*a*). The decrease in oxygen uptake of transgenic fish (open circles, broken line) and the control fish (solid diamonds, solid line) as they consumed the oxygen in the tank respirometer from hyperoxic levels to hypoxic levels. (*b*) The oxygen concentration at which fish lost equilibrium (i.e., were on their sides). Transgenic fish and control fish lost equilibrium at about the same oxygen concentration.



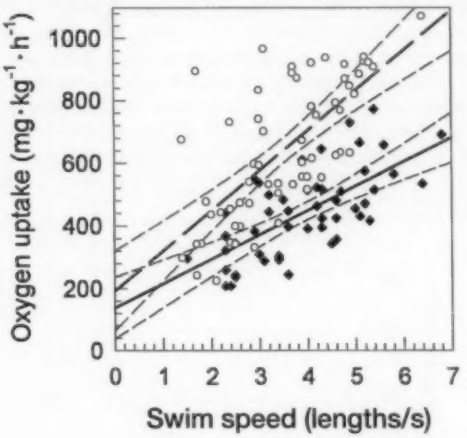
oxygen had decreased to 1.9 mg/L and 50% of the fish had lost equilibrium when it had decreased to 1.6 mg/L. At 1.6 mg/L the respirometers were flushed, but the fish continued to lose equilibrium, so there was a point when all fish were on their sides with complete loss of equilibrium. Although all fish lost equilibrium during the trial, all survived and were eating within 4 h of flushing the tank after the trial. Above the critical oxygen level, when oxygen concentration was above 10 mg/L, oxygen uptake was about 170 $mg \cdot kg^{-1} \cdot h^{-1}$ in control fish and 266 $mg \cdot kg^{-1} \cdot h^{-1}$ in transgenic fish; that is, oxygen uptake of transgenic fish was 1.6 times that of control fish.

Swimming respirometry

We were not successful at having the mean body weights of exactly 10 g in both groups at the same time because transgenic fish grew faster than anticipated. Therefore, the mean weight of the control fish (8.4 g) was slightly less than that of the transgenic fish (10.5 g). In addition, transgenic fish used in the swimming experiments were slightly longer and had lower condition factor than control fish (Table 2).

The oxygen uptake rate of transgenic fish was about 1.5–1.6 times that of control fish (Fig. 4). The equation of the line for 16 control fish was 138 + 78(speed) (r^2 = 0.46) and for 15 transgenic fish it was 193 + 128(speed) (r^2 = 0.48); oxygen uptake in milligrams O_2 per kilogram per hour and speed in body lengths (BL) per second. The difference between transgenic and control fish was statistically significant (analysis of covariance, with speed as the covariate, F = 80.73, $P < 0.001$). The slopes of the regression lines were significantly different (test for difference between slopes, F = 5.25 and $F_{critical0.05}$ = 3.93; P = 0.024). A logarithmic transformation of oxygen uptake did not improve the fit. The confidence limits for the intercept, oxygen uptake at zero swimming speed, overlap in part because this involves an extrapolation beyond measured points and in part because fish oxygen uptake at low swimming speeds tends to be extremely variable (Fry 1971; Stevens 1973).

Fig. 4. Oxygen uptake rates of transgenic (open circles) and control fish (solid diamonds) swimming at various rates in a Blazka swim tunnel. Regression lines (heavy broken line, transgenic fish; solid line, control fish) and 95% confidence intervals of the regression lines are shown (light broken lines).



Critical swimming speed

Mean critical swim speed did not differ between control and transgenic fish (Table 2; student's t test, P >0.05). Median critical swimming speed for control fish was 44.0 cm/s or 4.8 BL/s and for transgenic fish was 46.2 cm/s or 4.3 BL/s. Over the relatively small size range employed, critical swim speed (cm/s) did not correlate significantly either with body length, body weight, or condition factor.

Discussion

Growth

It has been known for some time that injections or implants of mammalian, avian, or fish GH all accelerate growth in salmonids (McLean and Donaldson 1993). However, the growth achieved with transgenic fish with endogenous GH release markedly exceeds that induced by injections or implants of exogenous GH (Du et al. 1992; Devlin et al. 1995). The different enhancement levels probably arise from the location and (or) number of copies of the gene insertion. For the present study we chose a transgenic line with an intermediate growth rate hoping to extend the experimental window in which transgenic and control fish of the same size would be available. Nevertheless, the growth rates of the transgenic line of fish that we used were excep-

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tional compared with those that can be achieved using exogenous GH. For example, our values for transgenic salmon are greater than the 62 values of salmonid growth rate in control fish and fish treated with hormones reported by Danzmann et al. (1990). However, they are not exceptional when compared with the maximal growth enhancement that has been achieved in transgenic salmon in other studies, which range from less than 2-fold to greater than 30-fold (Du et al. 1992; Devlin et al. 1995). Unfortunately, these authors did not report instantaneous growth rates, so direct comparison of growth rates is difficult. The body weight of the transgenic fish used in our experiments was 10-fold greater than that of control fish at 14 months of age.

Most previous experimenters studying the effects of growth hormone on salmonids have reported that injections or implants cause changes in external characteristics similar to smoltification; this also is the case in Atlantic salmon (Komourdjian et al. 1976). The role of growth hormone in evoking physiological changes associated with smoltification was first suggested by Ball (1969) and subsequently was shown by Sweeting et al. (1985). This size-dependent early smoltification appears to be an obligatory consequence of using fast-growing transgenic salmonids. Hence, all studies using transgenic salmonids are faced with the unavoidable confounding physiological factor of smoltification, which incidentally, also is in progress in nontransgenic salmon during the fall prior to the spring smolt migration.

Insofar as the present study is concerned, the most important difference between transgenic and control fish likely is the decrease in condition factor that occurs as the fish undergoes a rapid increase in length. Even at weights below 10 g, the specific line of transgenic fish that we used always showed a lower condition factor than control fish, so this attribute is not necessarily indicative of processes related to smolting. Moreover, in the present study, there was no significant correlation between condition factor and critical swimming speed.

Routine oxygen uptake

The diurnal pattern in oxygen uptake in both control and transgenic fish probably is related to feeding. Our methods do not permit us to isolate specific components, such as heat increment of feeding (i.e., specific dynamic activity), fish activity, or spurts in growth. Nevertheless, this relative difference and type of pattern is likely to be observed under commercial smolt production conditions, and future growers of such enhanced salmon should be prepared to either deliver more water or more oxygen in the water per unit biomass of transgenic fish compared with that required by nontransgenic salmon. This statement, however, is not meant to suggest that the total water or oxygen required per unit smolt production would necessarily be greater. Indeed, there are theoretical reasons (maintenance requirements compounded over less time; work in progress) to suggest that these production costs might be less as a result of the abbreviated production period required by the faster growing fish.

Oxygen dependancy of oxygen uptake

We are not aware of any studies other than ours that concern the limitations in oxygen uptake imposed by ambient oxygen tension in Atlantic salmon. Kutty and Saunders (1973) showed that swimming performance of Atlantic salmon is more highly dependent on oxygen concentration than is that of rainbow trout (*Oncorhynchus mykiss*), goldfish (*Carassius auratus*), or Tilapia (*Tilapia* spp.). The critical oxygen concentration (i.e., that at which the fish stops swimming) is 4.5 mg/L for Atlantic salmon compared with 2.0 mg/L for rainbow trout, 1.7 mg/L for goldfish, and 1.0 mg/L for Tilapia. The oxygen dependence of oxygen uptake has been measured in other salmonids. It is about 3.6 mg/L for standard metabolism in brook trout (*Salvelinus fontinalis*) at 15°C (Beamish 1964). During maximal swimming activity performance, maximal oxygen uptake is limited even at air saturation levels (Fry 1971). In the present experiments, critical oxygen uptake during routine activity was about 6 mg/L in transgenic fish and was about 4 mg/L in control fish; a result concordant with the observation that routine oxygen uptake is higher in transgenic fish.

Swimming respirometry

The oxygen uptake rates recorded in the present study are similar to those for salmonids.

Uptake values at 3 lengths/s for control (372 $mg \cdot kg^{-1} \cdot h^{-1}$) and transgenic (577 $mg \cdot kg^{-1} \cdot h^{-1}$) fish bracket the value of 457 $mg \cdot kg^{-1} \cdot h^{-1}$ reported for sockeye salmon (*Oncorhynchus nerka*; Brett 1964). However, comparisons across salmonid species may be questionable because oxygen uptake rates of postsmolt Atlantic salmon (200–850 g) reared under normal production conditions are 1.4–2.3 times higher than those for Pacific salmon (Fivelstat and Smith 1991). Because the slopes of the lines are different, our results suggest that transgenic fish have an increasingly higher cost of locomotion as U_{crit} is approached; we have no mechanistic explanation for this observation. The consequence is that the cost of locomotion to swim a given distance is greater in transgenic fish.

The standard metabolic rate (i.e., the intercept at zero swimming speed) of larger Atlantic salmon (226 g) is 111.2 $mg \cdot kg^{-1} \cdot h^{-1}$ (Withey and Saunders 1973). Extrapolating this value to 10 g, the approximate weight of our fish, using a weight exponent of 0.8 (Beamish 1978), yields a standard metabolic rate of 208 $mg \cdot kg^{-1} \cdot h^{-1}$, a value similar to our intercepts (Fig. 4). However, we do not place a great deal of confidence on extrapolated standard rates because of the large variablilty at low swimming speeds. For example, Brett (1964) reported that "restless" behaviour at low swimming speeds can have a large effect on metabolic rate in the swim tube; oxygen uptake rate in his study ranged from about 100 to 550 $mg \cdot kg^{-1} \cdot h^{-1}$.

Critical swimming speed

The critical swimming speeds of transgenic fish and control fish were not different from one another and are similar to literature values for salmonids of similar size (Beamish 1978). There are very few other values for Atlantic salmon, but ours are similar to those values that are available. Fifteen-centimetre fish in sea water at 11°C have a critical swimming speed of 54 cm/s or 3.6 BL/s (speed increments changed every 200 min) (Tang and Wardle 1992). Byrne et al. (1972) reported that Atlantic salmon (body weight about 200 g) swimming in a swim tunnel between 70 and 100 cm/s

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Table 3. Critical swim speeds of the present study on transgenic Atlantic salmon compared with the data reported by Farrell et al. (1997) on transgenic coho salmon.

	Length (cm)	Weight (g)	U_{crit} (cm/s)
Atlantic salmon (present study)			
Transgenic	10.8	10.5	44.2
Control	9.3	8.4	44.8
Coho salmon (Farrell et al 1997)			
Transgenic	22	121.6	47
Control 1	9.6	11	47
Control 2	20.1	86.1	69

for 2 h had a critical swim speed of "about 3–4 body lengths/s." In a similar type of test using 110-g Atlantic salmon, maximum speed sustained for several hours was 3.2 BL/s (Kutty and Saunders 1973). In a study concerning ration and swimming performance in 10-g rainbow trout, U_{crit} was not significantly different between fish on a 1% ration compared with those fed to satiation (Alsop and Wood 1997)

The only other critical swimming speed values for transgenic fish are for slightly larger coho salmon (*Oncorhynchus kisutch*; Table 3; Farrell et al. 1997). They reported that "transgenic fish swam much slower than older nontransgenic controls." They did not give a detailed history or details for the growth rates of their fish, but they did report that the transgenic fish were 10-fold heavier than control fish of the same age. Apparently the transgenic line they chose to test was one with an extremely enhanced growth rate (A.P. Farrell, Simon Fraser University, Burnaby, B.C., personal communication), whereas our transgenic line was one with a more modestly enhanced growth rate. Our results do not support their general conclusion that enhanced growth rate is associated with an obligatory diminished swimming capacity. Fast growth and high swimming performance are not necessarily mutually exclusive. Consider for example the extreme case of the tunas, which have extremely high growth and metabolic rates but also can sustain high swimming speeds. It is more likely that performance will be diminished only in transgenic fish exhibiting extremely enhanced growth rates. Fish with extremely enhanced growth rates are unlikely to be used in commercial production in any event, because they develop a number of other undesirable traits (Devlin et al. 1995).

There have been a number of studies that relate metabolic rate to food ration. For example, Beamish (1974) showed that the heat increment of feeding caused by a 4% ration was the same at three different swimming speeds. However, others have reported that it is higher at higher swimming speeds (Muir and Niimi 1972; Blaikie and Kerr 1996) or lower at higher swimming speeds (Furnell 1987). Our results suggest that the heat increment of feeding and (or) other physiological costs associated with high growth rates may be higher at higher swimming speeds.

Conclusions

Observations from the three different methods used to measure oxygen uptake in our study allow us to conclude that growth hormone transgenic salmon have a higher metabolic rate than nontransgenic salmon. Hogendoorn (1983) showed that there is a direct linear relation between the amount of oxygen consumed over a growth interval (not the rate of consumption) and the dry weight gain for catfish of different sizes and on a variety of ration levels. For the fish used in our experiments it took 20.5 days for the control fish to grow from 8 to 12 g (wet weight) and 11.3 days for transgenic to do the same. Assuming a similar water content for both groups of fish 8 and 12 g body weight, then based on Hogendoorns's findings we would predict that routine metabolic rate of transgenic fish would be 1.8 times that of control fish. This value is similar to our observations (routine metabolic rate of transgenic fish was 1.70 times that of control fish). Therefore, GH-enhanced transgenic salmon appear to exibit the same positive relationship between routine feeding, growth, and metabolic rates as might be expected from non enhanced fish but with an extended scope as a result of their compressed life history.

It also has been reported that exogenous ovine GH can cause an increase in oxygen uptake in Atlantic salmon: about a 30% increase in presmolts and a 16% increase in parr (Seddiki et al. 1996). Our study extends these observations to endogenous GH production. In addition, it has been shown that restricting ration to 1% of body mass daily causes a reduction in routine oxygen uptake and a decrease in oxygen uptake at all swimming speeds relative to fish fed to satiation (Alsop and Wood 1997). These workers also argued that, because the heat increment of feeding will be larger in fish fed larger rations, the larger heat increment of feeding should reduce U_{crit} because there will be less oxygen available for swimming. A major difference between their study on ration level and our study on transgenic fish is that our fish ate more from the day of first feeding, and we suspect that our transgenic fish have adapted to the larger ration with adaptations in the digestive, respiratory, and cardiovascular systems. We currently are attempting to measure these parameters. Alsop and Wood altered ration level for only 17 days. Perhaps 17 days in high ration is not sufficient time to allow for adaptation of the systems involved in the acquisition and delivery of oxygen.

Future work is necessary to define the efficiencies and incorporate these into "cost of production models" before the full benefits of using such growth enhanced fish in commercial farming can be realized. We conclude that these GH transgenic fish have a higher oxygen uptake during routine activity and during forced swimming activity than similar-sized control fish, but do not differ in regards to critical swimming speed.

Acknowledgements

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Methyl mercury in aquatic insects from an experimental reservoir[1]

B.D. Hall, D.M. Rosenberg, and A.P. Wiens

Abstract: Our objective was to study the effects of experimental flooding of a small wetland lake on the methyl mercury (MeHg) concentrations in aquatic insects and to compare MeHg concentrations in insects with those in water and fish from the same system. Insects were collected from the shorelines of the experimental reservoir before and after flooding, an undisturbed wetland lake, and an oligotrophic lake, all in the Experimental Lakes Area in northwestern Ontario. Samples were identified to the lowest possible taxon and categorized into functional feeding groups (FFGs; predators or collector/shredders). The insects were analyzed for MeHg and total Hg using clean techniques. Contamination was not a problem because levels of MeHg in insects were much higher than background concentrations. Odonata, Corixidae, Gerridae, Gyrinidae, and Phryganeidae/Polycentropodidae exhibited increases in MeHg concentrations in response to flooding. When data were grouped into FFGs, increases were observed in predators. There were insufficient numbers of collector/shredders collected to make a definitive conclusion on MeHg increases. Predators exhibited an approximately threefold increase in MeHg concentrations after flooding compared with a 20-fold increase in water concentrations and a four- to five-fold increase in fish concentrations. Trends in MeHg concentrations in aquatic insects from reservoirs and natural lakes in Finland and northern Québec were similar to ours. Evidence of an increase in MeHg concentrations in the lower food web helps explain increases in MeHg concentrations in fish from reservoirs because food is the major pathway of MeHg uptake in fish tissue.

Résumé : Notre objectif était d'étudier les effets d'une inondation expérimentale d'un petit lac palustre sur les concentrations de méthyl mercure (MeHg) dans les insectes aquatiques, et de comparer les concentrations de MeHg dans les insectes à celles présentes dans l'eau et les poissons du même système. On a recueilli les insectes sur les rives du réservoir expérimental avant et après l'inondation, d'un lac palustre non perturbé et d'un lac oligotrophe, ces trois plans d'eau se trouvant dans la Région des lacs expérimentaux, dans le nord-ouest de l'Ontario. Les insectes ont été identifiés au plus bas taxon possible et groupés selon leur type d'alimentation (prédateurs ou collecteurs/broyeurs). On a dosé le MeHg et le Hg total dans les insectes au moyen de techniques propres. On n'a pas eu de problème de contamination étant donné que les niveaux de MeHg dans les insectes étaient beaucoup plus élevés que les concentrations de fond. Il y a eu accroissement des concentrations de MeHg chez les Odonates, les Corixidés, les Gerridés, les Gyrinidés et les Phryganéidés/Polycentropodidés par suite de l'inondation. En groupant les données selon les types d'alimentation, on a observé des accroissements chez les prédateurs. Le nombre insuffisant de collecteurs/broyeurs recueillis ne nous a pas permis de conclure avec certitude qu'il y a eu dans ce groupe accroissement du MeHg. Les concentrations de mercure dans les prédateurs se sont accrues d'un facteur 3 environ après l'inondation, tandis que les concentrations dans l'eau et dans les poissons se sont accrues d'un facteur 20 et d'un facteur 4 ou 5, respectivement. On a observé des tendances des concentrations de MeHg similaires aux nôtres dans des insectes aquatiques de réservoirs et de lacs naturels de la Finlande et du Québec. Les preuves de cet accroissement des concentrations du MeHg dans les échelons inférieurs du réseau alimentaire aident à expliquer les accroissements des concentrations de MeHg dans les poissons des réservoirs, étant donné que l'alimentation est la voie d'entrée principale du MeHg dans les tissus des poissons.

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Introduction

Methyl mercury (MeHg) is a known neurotoxin that can be a byproduct of either industrial activity or reservoir creation for power production (e.g., Wheatley and Paradis 1995). For example, concentrations of MeHg in fish harvested from northern Canadian reservoirs often exceed Canadian marketing limits of 0.5 g·g wet mass^{-1} (Bodaly et al. 1984; Verdon et al. 1991; Morrison and Thérien 1995). Thus, the health and well-being of people who depend on reservoir fisheries may be threatened (Rosenberg et al. 1995).

The threat posed by MeHg to human populations has spawned a great deal of research into its biogeochemistry

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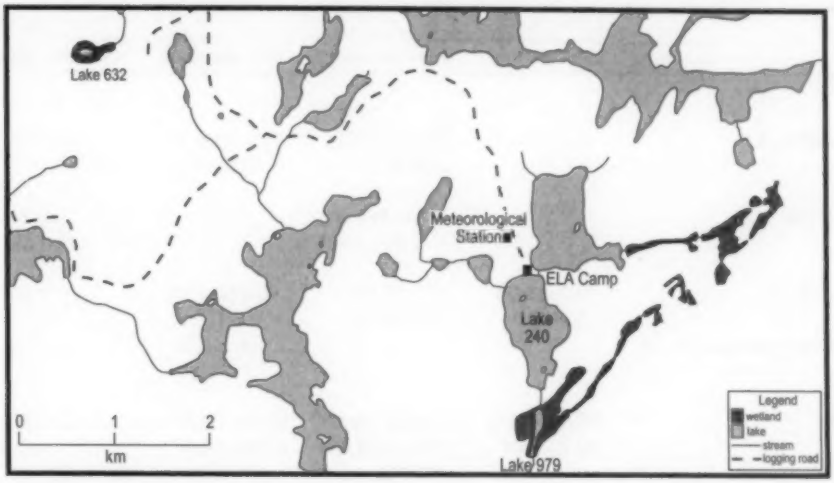
Fig. 1. Location of L979 and L632 in the ELA, northwestern Ontario (adapted from St. Louis et al. 1996).



and movement through the food web. The development of analytical techniques sensitive enough to measure low levels of MeHg in water (Bloom 1989) has led to improved understanding of the biogeochemical cycling of MeHg in aquatic environments such as natural wetlands (e.g., St. Louis et al. 1994, 1996), flooded wetlands (e.g., Kelly et al. 1997), and large-scale hydroelectric reservoirs (e.g., Dmytriw et al. 1995). The importance of dietary sources of MeHg to fish has been clarified by experiments in the field (Hall et al. 1997), experiments in the laboratory (Phillips and Buhler 1978; Rodgers and Beamish 1981), and theoretical models (Harris and Snodgrass 1993; Rodgers 1994). It has become apparent that MeHg concentrations in lower trophic levels are important in determining concentrations of MeHg in fish.

Hg dynamics in aquatic insects have been previously examined primarily in laboratory studies using unnaturally high levels of total mercury (THg) and MeHg (Rossaro et al. 1986; Saouter et al. 1993) or in field studies that have measured only THg (Parkman and Meili 1993; Snyder and Hendricks 1995). One study has dealt with MeHg in natural systems (Tremblay et al. 1996*a*) and three studies have dealt specifically with MeHg concentrations in reservoirs (Surma-Aho et al. 1986; Tremblay et al. 1996*b*; Tremblay and Lucotte 1997). The latter two compared MeHg concentrations in aquatic insects from reservoirs and natural lakes.

The research for our study was a component of the Experimental Lakes Area Reservoir Project (ELARP), a whole-ecosystem study designed to develop the understanding of two problems associated with the decomposition of flooded organic matter caused by reservoir creation: (*i*) increased MeHg concentrations in fish harvested from reservoirs and (*ii*) increased fluxes of greenhouse gases (CO_2 and CH_4) from reservoirs created over peatlands (Kelly et al. 1997).

Our objective was to quantify changes in THg and MeHg concentrations in aquatic insects before and after the creation of an experimental reservoir. Although THg analyses were done, we emphasized MeHg dynamics because MeHg is the biologically active form (Kelly et al. 1997). We posed two questions: (*i*) does flooding a wetland lake affect the concentrations of MeHg in aquatic insects? and (*ii*) how do increases in MeHg concentrations in insects compare with increases in water and fish?

Materials and methods

Site descriptions

Two wetlands at the Experimental Lakes Area (ELA) in northwestern Ontario were chosen as study sites (Fig. 1). The experimentally flooded wetland, Lake 979 (L979), had a 2.4-ha central pond surrounded by a 14.2-ha peatland; its main inflow originated from upstream, oligotrophic Lake 240 (L240). L979 was initially impounded in late June 1993 by damming its outflow (Kelly et al. 1997). As a result, surface area of the lake increased to 16.7 ha and maximum depth increased from about 1 to 2.3 m. To simulate operation of boreal hydroelectric reservoirs, L979 was drawn down by 1 m in early October. A similar cycle of flooding and drawdown was followed in 1994. The reference wetland, Lake 632 (L632), is a headwater system with a 0.86-ha central pond and a surrounding peatland area of 3.4 ha. St. Louis et al. (1996) described the two wetland systems in more detail.

Sampling program

A nonquantitative sampling program was established along the shorelines of both L979 and L632. Insects were sampled by sweeping with a triangular (32 × 34 × 32 cm) net of 400-μm mesh size. Sampling was done until sufficient biomass for at least one MeHg and one THg analysis was obtained for each major group (see Analysis of Hg in insects section below) or until about 16 h of time was spent sampling. L979 was sampled before and after it was flooded (every 2 weeks during May–September 1992 and 1993 and weekly during 2–22 June 1993). After flooding, sampling was relocated away from the edge of the old lake to shallow areas over newly flooded peat. Samples in 1994 were collected starting on 18 May before reflooding (26 May 1994) and every 2 weeks after reflooding until 1 September 1994. A single sample was taken from the former shoreline after drawdown in mid-October 1994. Shoreline insects were collected about every 2 weeks in L632 from May to September 1992–1994. Samples were also periodically taken from L240 (an upstream reference to L979) in 1993 and 1994 (10 May – 24 August 1993 and 8–9 June 1994).

Samples were sorted into major taxa in the field and were identified to lower taxa (usually genus) in the laboratory using keys

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Table 1. Concentration of MeHg in insects and other invertebrates sampled from L979, L632, and L240.

Taxon[a]	L979				
	1992 preflood	1993 preflood	1993 postflood	1994 drawdown	1994 reflood
Individual taxa					
Amphipoda					
Hyalella azteca (C/S, A)	67.7 (1)	78.2±12 (2) 65.9–90.5			128.4±21 (5) 54.3–188.0
Ephemeroptera					
Siphlonurus sp. (C/S, N)		87.1 (1)			
Trichoptera					
Limnephilidae (C/S, L)	24.7±13 (3) 3.4–49.0	47.7±16 (5) 19.0–107.8	46.8 (1)		
Phryganeidae, Polycentropodidae (P, L)					86.0 (1)
Odonata					
Aeshnidae (P, N)	107.3±17 (10) 60.1–196.6	79.8±17 (4) 49.7–121.4	191.3±13 (2) 178.0–204.5		169.4±41 (5) 19.4–234.6
Corduliidae (P, N)		59.4 (1)		200.3 (1)	179.0±26 (5) 136.6–278.3
Other Odonata (P, N)	88.2±0.1 (2) 88.1–88.2	79.3±5 (3) 69.6–87.5	283.1±141 (3) 11.4–484.3	84.7 (1)	195.7±51 (5) 56.2–359.5
Total Odonata (P, N)	104.1±15 (12) 60.1–196.6	77.1±9 (8) 49.7–121.4	246.4±81 (5) 11.4–484.3	142.5±58 (2) 84.7–200.3	181.3±22 (22) 19.3–359.5
Hemiptera					
Belostomatidae					
Lethocerus americanus (P, A)	499.3±28 (3) 443.1–531.2	107.7 (1)			
Corixidae (P, A, N)			318.1±33 (6) 221.8–462.8	285.4±3 (2) 282.6–288.3	157.1±17 (6) 99.6–210.7
Gerridae					
Gerris sp. (P, A, N)		108.6 (1)	404.9±118 (3) 200.8–610.7		180.6±104 (2) 76.6–284.5
Notonectidae					
Notonecta sp. (P, A, N)		159.6±134 (2) 25.9–293.4	260.6±28 (5) 183.9–336.0	331.4 (1)	442.7±60 (15) 86.3–845.8
Nepidae					
Ranatra sp. (P, A)					
Coleoptera					
Dytisicidae					
Dytiscus sp. (P, A)	216.1±19 (15) 50.3–332.6	118.2 (1)	187.5 (1)	337.8 (1)	169.2±62 (3) 60.9–272.4
Gyrinidae					
Gyrinus sp. (P, A)	19.0±6 (2) 13.1–24.5	34.6±9 (2) 25.5–43.7		84.4 (1)	106.0±12 (7) 59.1–146.8
Dineutus sp. (P, A)	29.6 (1)	39.8±4 (2) 35.6–44.0			169.1 (1)
Total Gyrinidae (P, A)	35.1±11 (5) 13.1–78.6	55.4±13 (6) 25.5–107.9		84.4 (1)	113.9±13 (8) 59.1–169.1
Others (P)[b]		54.7 (1)	53.8 (1)		196.0±40 (10) 50.1–377.0
Functional feeding groups					
Total collector/shredders	35.4±14 (4) 24.7–67.7	60.3±12 (8) 47.7–87.1	46.8 (1)		128.4±21 (5) 54.3–188.0
Total predators	176.1±23 (35) 13.1–351.2	82.9±13 (20) 25.5–293.4	270.1±31 (21) 11.4–610.7	229.9±41 (7) 84.4–331.4	233.7±23 (59) 50.1–846.0

Note: The top number is the MeHg concentration (ng·g d.m.$^{-1}$) ± 1 SE; the bottom numbers are the range of MeHg concentrations. The number of analyses is given in parentheses.

[a]C/S, collector/shredders; P, predators; A, adults; L, larvae; N, nymphs.

[b]Other Coleoptera predators include Gyrinidae larvae, other Dytiscidae adults, and larvae not including *Dytiscus* sp.

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L979	L632			L240	
1994 drawdown	1992	1993	1994	1993	1994
	31.0 (1)	57.4±21 (6) 11.7–126.9	18.9 (1)		
	48.3±23 (2) 25.2–71.3		136.3±9 (6) 108.1–168.8		
	26.4 (1)	37.6±15 (7) 1.3–124.8			
351.5±94 (5) 126.4–694.0	35.1 (1)	32.7±5 (8) 9.0–51.1			
344.7±154 (3) 132.9–645.2	135.6±20 (2) 115.5–155.7	77.3±6 (32) 34.6–182.0	185.8±21 (6) 139.5–266.6		
216.0±38 (4) 133.2–282.6	95.7 (1)	87.3±8 (18) 48.4–139.5 72.6±9 (11) 7.6–116.1	140.0±22 (13) 60.0–361.8 86.2±18 (9) 21.2–173.7		
271.2±67 (7) 132.9–645.2	122.3±33 (3) 95.7–155.7	79.1±4 (61) 7.6–182.0	132.5±14 (28) 21.2–361.8	52.6±11 (8) 14.2–112.1	112.9±10 (3) 93.7–129.3
	429.2±43 (32) 71.9–1022.7 121.0 (1)	310.6±74 (7) 139.6–600.0 155.8±22 (3)	93.5±5 (4) 86.1–109.6 124.2±22 (8)		
233.7. (1)		113.1–184.2	36.9–218.1		
		142.2±47 (2) 94.7–189.7		118.6±7 (6) 86.5–136.3	151.0±12 (2) 139.5–162.5
715.5 (1)	430.2 (1)	182.3±25 (7) 65.7–264.9	235.0±50 (10) 96.0–612.6		
	334.4±1 (2) 333.0–335.8	233.7 (1)	365.5±137 (2) 228.9–502.2		
	196.0±105 (3) 10.4–375.5	204.7±33 (11) 96.5–487.2	156.3±30 (9) 30.2–281.6		
		55.0±17 (2) 38.5–71.6	64.7±10 (3) 46.0–78.5 172.7 (1)		
	68.0±15 (4) 44.5–107.2	55.0±17 (2) 38.5–71.6 307.0 (1)	91.7±28 (4) 46.0–172.7 88.1 (1)	57.0±6 (8) 32.8–82.9	
	38.4±11 (4) 25.2–71.3	46.7±13 (13) 1.3–127.0	119.5±19 (7) 18.9–168.8		
344.0±57 (9) 126.4–715.5	339.4±35 (46) 44.5–1022.7	125.0±11 (95) 7.6–600.0	152.4±13 (66) 21.2–502.2	86.5±14 (22) 14.2–136.3	128.1±12 (5) 93.7–162.5

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Table 2. Significant differences (ANOVA, $p < 0.05$) in MeHg concentrations among taxa and functional feeding groups of aquatic insects using state of flooding, lake, and year as main effects.

	Main effect											
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	
	Lake 979						Lake 632			Lake 240		
	1992 preflood	1993 preflood	1993 postflood	1993 drawdown	1994 reflood	1994 drawdown	1992	1993	1994	1993	1994	*p*-value
Individual taxa												
Ephemeroptera, overall ANOVA												0.012
1. L632 1992 vs. L979 1993 preflood	nd	1	nd	nd	nd	nd	–1	nd	0	nd	nd	0.249
2. L632 1992 vs. L632 1994	nd	0	nd	nd	nd	nd	1	nd	–1	nd	nd	0.021
Trichoptera, overall ANOVA												0.007
Phryganeidae, Polycentropodidae												
3. L979 flooded vs. L632 1992 and 1993	nd	nd	nd	nd	–1	–1	1	1	nd	nd	nd	0.048
Odonata, overall ANOVA												<0.001
4. L979 preflood vs. natural lakes	5	5	0	0	0	0	–2	–2	–2	–2	–2	0.571
5. L979 1992 vs. L979 1993 preflood	–1	1	0	0	0	0	0	0	0	0	0	0.128
6. L979 preflood vs. L979 postflood	–2	–2	1	1	1	1	0	0	0	0	0	<0.001
Hemiptera												
Belostomatidae												
Lethocerus americanus, overall ANOVA												0.035
7. L979 preflood vs. L632	–3	–3	nd	nd	nd	nd	2	2	2	nd	nd	0.851
8. L632 1992 vs. L632 1993 and 1994	0	0	nd	nd	nd	nd	–2	1	1	nd	nd	0.006
Corixidae, overall ANOVA												<0.001
9. L979 flooded vs. L632	nd	nd	3	3	3	3	–4	–4	–4	nd	nd	0.001
Gerridae												
Gerris sp., overall ANOVA												0.045
10. L979 1993 preflood vs. L240	nd	–2	0	nd	0	nd	nd	0	nd	1	1	0.435
11. L979 1993 preflood vs. 1993 postflood	nd	–1	1	nd	0	nd	nd	0	nd	0	0	0.043
Notonectidae												
Notonecta sp., overall ANOVA												0.013
12. L979 1993 preflood vs. L632	nd	–3	0	0	0	0	1	1	1	nd	nd	0.338
13. L979 1993 preflood vs. L979 postflood	nd	–4	1	1	1	1	0	0	0	nd	nd	0.057
Nepidae												
Ranatra sp., overall ANOVA												0.039
14. L632 1992 vs. L632 1994	nd	nd	nd	nd	nd	nd	1	0	–1	nd	nd	0.025
15. L632 1992 and 1994 vs. L632 1993	nd	nd	nd	nd	nd	nd	1	–2	1	nd	nd	0.142
Coleoptera												
Gyrinidae, overall ANOVA												0.005
16. L979 preflood vs. natural lakes	2	2	nd	0	0	nd	–1	–1	–1	–1	nd	0.090
17. L979 1992 vs. L979 1993 preflood	–1	1	nd	0	0	nd	0	0	0	0	nd	0.132
18. L632 1992 vs. L632 1994	0	0	nd	0	0	nd	–1	0	1	0	nd	0.287
19. L979 preflood vs. L979 postflood	–1	–1	nd	1	1	nd	0	0	0	0	nd	0.010

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Table 2 (*concluded*).

	Main effect											
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	
	Lake 979						Lake 632			Lake 240		
	1992 preflood	1993 preflood	1993 postflood	1993 drawdown	1994 reflood	1994 drawdown	1992	1993	1994	1993	1994	*p*-value
Functional feeding groups												
Total collector/shredders, overall ANOVA												0.005
20. L632 1994 vs. L632 1992 and 1993	0	0	0	nd	0	nd	–1	–1	2	nd	nd	0.001
21. L979 1993 preflood vs. L979 reflooded	0	–1	0	nd	1	nd	0	0	0	nd	nd	0.015
Total predators, overall ANOVA												<0.001
22. L979 1993 preflood vs. L632 1993, L632 1994, and L240	0	–4	0	0	0	0	0	1	1	1	1	0.576
23. L632 1992 vs. L632 1993 and 1994	0	0	0	0	0	0	–2	1	1	0	0	<0.001
24. L632 1993 vs. L632 1994	0	0	0	0	0	0	0	–1	1	0	0	0.129
25. L240 1993 vs. L240 1994	0	0	0	0	0	0	0	0	0	–1	1	0.576
26. L979 1993 preflood vs. 1993 postflood	0	–1	1	0	0	0	0	0	0	0	0	<0.001
27. L979 1993 preflood vs. 1993 drawdown and 1994	0	3	0	–1	–1	–1	0	0	0	0	0	<0.001

Note: Orthogonal contrasts (numbered 1–27) were used to identify the source of any differences. Within an orthogonal contrast, elements (i.e., average MeHg concentrations) with the same sign are compared with elements of the opposite sign; the total of each sign must balance. Elements not compared are assigned 0; nd, no data. For example, under "Individual taxa, Coleoptera, Gyrinidae" contrast 19 compared MeHg concentrations in Gyrinidae in L979 in the two preflood years (columns I and II: $-1 + -1 = -2$) with postflood conditions (columns IV and V: $1 + 1 = 2$). It was not always possible to perform the most desirable contrasts because of a lack of data.

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from Merritt and Cummins (1984). The large number of specimens collected made it impossible to allow a period of gut depuration, so specimens were frozen soon after sorting. It was assumed that the total body burden of MeHg is potentially transferable to fish, although this may not always be true (Hare 1992).

Insects were classified into functional feeding groups (FFGs) according to food type and feeding habit (Merritt and Cummins 1984). Three FFGs resulted initially: (*i*) predators: insects feeding on other invertebrates, (*ii*) collector/shredders: insects feeding on living plants or detritus, and (*iii*) predator/herbivores: insects feeding on both other animals and plants or insects that change their diet at different life stages. However, only two FFGs were used (predators and collector/shredders) when stable nitrogen isotope analysis (Peterson and Fry 1987; Hesslein and Ramlal 1993) revealed no difference between predator and predator/herbivore groups (B.D. Hall, unpublished data).

Analysis of Hg in insects

Insects were rinsed in low-Hg water, blotted to remove excess water, and placed individually into acid-washed plastic scintillation vials. A minimum of 0.05 g dry mass (d.m.) of sample was required for MeHg and THg analysis. Insects of the same taxa were often pooled on the same sampling date to obtain sufficient biomass for analysis. Samples were freeze-dried, weighed, and then ground using an acid-washed glass mortar and pestle. Contamination was not a problem because levels of MeHg in insects were much higher than background concentrations.

Organic Hg and THg were determined using cold-vapour atomic absorption spectrophotometry (AAS; Armstrong and Uthe 1971; Hendzel and Jamieson 1976; Malley et al. 1996). Ground, dried samples for MeHg analysis were homogenized in $CuSO_4$ and NaBr. Organic Hg was extracted into a 3:2 methylene chloride – hexane solution. An aliquot of the organic fraction was completely digested in HNO_3 and H_2SO_4 overnight. Reference material (National Research Council of Canada dogfish muscle (Dorm-1)) was analyzed along with samples; organic Hg determinations of the reference material were always within the certified range (671–793 ng·g d.m.$^{-1}$). Two samples analyzed for organic Hg in our laboratory were also analyzed at the ELA MeHg laboratory using distillation, ethylation, and detection by atomic fluorescence (Horvat et al. 1993, with modifications given in Moore et al. 1995); concentrations of MeHg in these samples (452 and 180 ng·g d.m.$^{-1}$) were within the range of those found by the AAS method (423 and 228 ng·g d.m.$^{-1}$). These results confirmed that the organic Hg extracted by the AAS method was primarily MeHg (A. Heyes, McGill University, Montréal, Qué., personal communication). The high cost of the confirmation technique prohibited the analysis of more than two samples.

Sources of variability for the MeHg techniques used here are not usually available; however, we were able to partition coefficients of variation (CVs) into analytical error associated with instruments (2%), analytical error associated with sample preparation (homogenization and digestion: 9%), and natural variation among individuals (24%). Instrument error was determined by repetitive analysis of subsamples of the same extraction digest on the same day. Sample preparation error was determined by analyzing different extraction digests of a sample on the same day. Natural variation was determined by analyzing different sets of samples of the same taxon collected on the same date. The overall CV of 35% was originally regarded as natural variation, and the constituent variations were obtained by simple subtraction. Overall CVs of 40% are considered acceptable in most toxicological studies (Chapman 1991). Concentrations of THg and MeHg reported here are uncorrected for overall error.

Statistical analysis

Simple arithmetic means were calculated for individual samples, averaged, and differences tested using analysis of variance (ANOVA) or *t*-tests (SYSTAT, Wilkinson 1990). Caution was used in interpreting significance levels because of temporal pseudoreplication (Hurlbert 1984). When the null hypothesis (H_0 = no change in MeHg concentrations in aquatic insects in response to flooding) was rejected at the 5% level, orthogonal contrasts were used to determine which main effects were significantly different in individual taxa. Orthogonal contrasts examined whether significant differences in MeHg concentrations were caused by interlake variation, annual variation, or flooding effect. FFGs were compared using *t*-tests to determine if they accumulated different concentrations of MeHg. Data for each taxon and FFG were organized by lake, year, and state of flooding for comparison.

MeHg concentrations in insects and water and THg concentrations in fish (finescale dace, *Phoxinus neogaeus*) were compared visually rather than statistically because different sampling intervals were used to collect data from each environmental category. Concentrations of MeHg in the FFGs were compared with MeHg data in unfiltered water (analyzed using clean techniques and intralaboratory comparisons; see Kelly et al. 1997) and THg concentrations (analyzed using AAS) in fish held in pens (Bodaly and Fudge 1994).

Results

Average MeHg concentrations for each taxon in each lake are presented in Table 1. Annual averages are presented for L632 and L240 whereas data were grouped according to state of flooding for L979. The average MeHg concentration in predators (mean = 189.0 ng·g d.m.$^{-1}$, range = 19.0–715.5 ng·g d.m.$^{-1}$, n = 399) was significantly greater than the average MeHg concentration in collector/shredders (mean = 71.5 ng·g d.m.$^{-1}$, range = 18.9–136.0 ng·g d.m.$^{-1}$, n = 36) (*t*-tests, $p < 0.001$).

Significant ANOVAs ($p < 0.05$) are shown in Table 2. Further examination of these significant ANOVAs using orthogonal contrasts often revealed the source of the differences. For example, under "Individual taxa, Coleoptera" the overall ANOVA done for Gyrinidae was significant (p = 0.005). Was the significant test result caused by lake-to-lake differences (contrast 16; Table 2) or flooding effects (contrast 19; Table 2)? Only contrast 19 was significant ($p <$ 0.05), indicating that a flooding effect was responsible. The caption to Table 2 explains how to use the orthogonal contrasts; the results of these comparisons are presented next.

Differences in MeHg concentrations as a result of lake-to-lake variation

MeHg concentrations in benthic invertebrates did not vary significantly between L979 in its natural state and reference L632 and L240. Tests for lake-to-lake differences in Ephemeroptera, Odonata, Belostomatidae, Gerridae, Notonectidae, Gyrinidae, and the predator FFG (contrasts 1, 4, 7, 10, 12, 16, and 22; Table 2) were nonsignificant ($p > 0.05$).

Differences in MeHg concentrations as a result of annual variation within lakes

Annual variability in L979 was determined by comparison of preflood concentrations from 1992 and 1993 (contrasts 5 and 17). In the reference lakes, comparisons were made between concentrations in different years within the same lake (L632: contrasts 2, 8, 14, 15, 18, 20, 23, and 24; L240: contrast 25).

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Contrasts used to test annual variability showed no significant preflood differences in L979 (contrasts 5 and 17). There were no significant differences due to annual variation in L240 (contrast 25). No differences were found in L632 Nepidae (contrast 15) or Gyrinidae (contrast 18) in 1992 compared with 1993 and 1994. Significant year-to-year differences were evident in some taxa from L632 (contrasts 2, 8, 14, 20, and 23). For example, contrast 8 showed that 1992 MeHg concentrations in *Lethocerus americanus* were significantly higher than in other years. High concentrations of MeHg in *L. americanus* in L632 in 1992 affected the results of contrast 23, done for the predator FFG, and affected the mean predator concentrations for 1992 in both L632 (339.4 ng·g d.m.$^{-1}$) and L979 (176.1 ng·g d.m.$^{-1}$) (Table 1). Concentrations of MeHg in *L. americanus* in subsequent years were lower, as were numbers collected (Table 1); contrast 24 shows no significant difference between 1993 and 1994.

Contrast 14 for *Ranatra* also showed that MeHg concentrations differed among 1992 and 1994 in L632. High variability within the 1994 samples may have caused this result (Table 1).

Contrast 20 showed a significant difference in MeHg concentrations for collector/shredders in 1994 compared with 1992 and 1993. In 1994, concentrations in L632 and L979 collector/shredders increased significantly from 1992 and 1993 (Fig. 2A). Most (85%) of the collector/shredders in L632 in 1994 were late-instar *Siphlonurus* that had relatively high MeHg concentrations. The other collector/shredder present in L632 in 1994 (*Hyalella azteca*) did not have high MeHg concentrations. *Siphlonurus* was not present in L979.

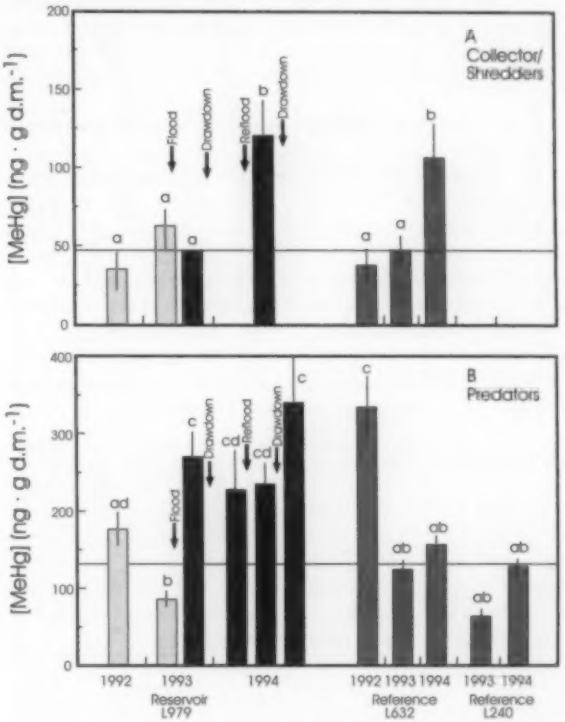
Annual variation in L632 suggests that increased concentrations in L979 may be attributable to year-to-year differences. However, the effects of flooding (see next section) were much greater than those attributable to annual variation.

Differences in MeHg concentrations as a result of flooding of L979

Orthogonal contrasts used to test flooding effect included all taxa that were present in L979 before and after flooding, except Ephemeroptera, Belostomatidae, and Nepidae. The relevant contrasts included either MeHg concentrations from L979 before and after flooding (contrasts 6, 11, 13, 19, 21, 26, and 27) or concentrations in flooded L979 compared with the reference lakes (contrasts 3 and 9). The contrasts using individual taxa (contrasts 3, 6, 9, 11, 13, and 19) revealed significant effects on Phryganeidae/Polycentropodidae (contrast 3), Odonata (contrast 6), Corixidae (contrast 9), Gerridae (contrast 11), and Gyrinidae (contrast 19). Notonectidae (contrast 20) did not exhibit significant increases in MeHg concentrations after flooding.

Differences in MeHg concentrations in FFGs before and after flooding were also apparent (contrasts 21, 26, and 27). MeHg concentrations in L979 predators increased after flooding (contrasts 26 and 27; Fig. 2B) and were still elevated at the end of the study. The first significant increase in MeHg concentrations in collector/shredders in L979 occurred after reflooding in 1994 (contrast 22; Fig. 2A), although this increase coincided with increased concentrations in L632 (contrast 20). In all, eight out of 11 contrasts (contrasts 3, 6, 9, 11, 19, 21, 26, and 27) in five out of six taxa examined showed increased MeHg after flooding of L979.

Fig. 2. MeHg concentrations ±SE in functional feeding groups of aquatic insects: (A) predators and (B) collector/shredders. Bars with the same letter within each panel are not statistically different (see Table 1). The horizontal line represents average MeHg concentrations before flooding (1992 and 1993). Light bars indicate preflood concentrations in L979, dark bars indicate postflood concentrations, and medium bars represent concentrations in reference systems (L632 and L240).



Concentrations of MeHg in water, aquatic insects, and fish

Concentrations of MeHg in unfiltered water from L979 (Kelly et al. 1997) and MeHg concentrations in the two invertebrate FFGs are shown in Fig. 3. Average end of season THg concentrations (THg ≈ MeHg in fish) in omnivorous fish held in pens in L979 ranged from 0.069 to 0.139 μg·g^{-1} in preflood conditions and from 0.220 to 0.300 μg·g^{-1} in postflood conditions (R.A. Bodaly, Freshwater Institute, Winnipeg, Man., personal communication). Concentrations of MeHg before and after flooding increased 20-fold in water, about three-fold in predatory insects, and two- to three-fold in fish.

Discussion

Effect of flooding on concentrations of MeHg in aquatic insects

Summary of the ELA data

The quantitative trends of MeHg concentrations in inver-

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Fig. 3. Concentrations of MeHg in (A) water, (B) collector/shredder insects, and (C) predatory insects from L979. Water data are from Kelly et al. (1997). Note scale differences.

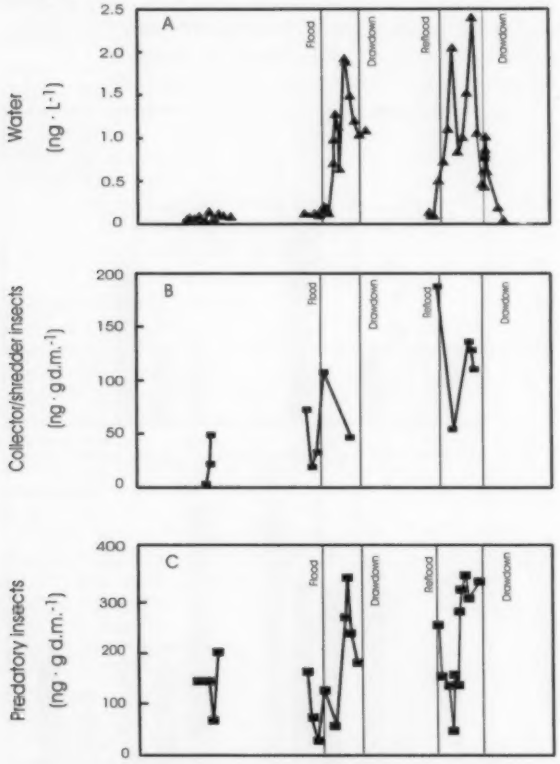

Table 3. Changes in concentrations of MeHg in insects and other invertebrates in response to flooding of L979.

Taxon	Life stage	Increased	No change
Collector/shredders			
Amphipoda			
Hyalella azteca	Adults		+
Trichoptera			
Limnephilidae	Larvae		+
Predators			
Odonata			
Aeshnidae	Nymphs	+	
Corduliidae	Nymphs	+	
Other Odonata	Nymphs	+	
Hemiptera			
Corixidae	Adults, nymphs	+[a]	
Gerridae			
Gerris sp.	Adults, nymphs	+	
Notonectidae			
Notonecta sp.	Adults, nymphs		+
Coleoptera			
Dytiscidae			
Dytiscus sp.	Adults		+
Gyrinidae			
Gyrinus sp.	Adults	+	
Dineutus sp.	Adults	+	
Trichoptera			
Phryganeidae, Polycentropodidae	Larvae	+[a]	

[a]Where L979 preflood data were not collected, L979 MeHg concentrations were compared with those in L632.

tebrates, as summarized from Tables 1 and 2, are shown in Table 3. When examining the data at the genus or family level, MeHg concentrations either increased in response to flooding of L979 or showed no change; no groups showed a decrease in MeHg concentrations in response to flooding. Odonata, Corixidae, Gerridae, Gyrinidae, and Phryganeidae/Polycentropodidae exhibited increases in MeHg concentrations in response to flooding. There were significant increases in MeHg in predators within a few months of flooding in 1993 (82.9 ng·g d.m.$^{-1}$ before flooding versus 270.1 ng·g d.m.$^{-1}$ after flooding). Concentrations in predators were elevated a year after flooding, indicating that effects of flooding last longer than one season. MeHg concentrations also remain elevated for prolonged periods in fish from reservoirs (Bodaly et al. 1984; Anderson et al. 1995). The pattern of MeHg increase was not observed in invertebrate collector/shredders. Although the 1994 level differed significantly from previous years in L979 (Fig. 2A), concentrations in collector/shredders were also high in L632 in 1994. In general, MeHg concentrations in predators from L240 and L632 did not differ significantly from L979 before flooding.

Comparisons with other studies

Trends observed in changes in MeHg concentrations in response to flooding by aquatic insects at the ELA were similar to results of studies in Finland (Surma-Aho et al. 1986) and northern Québec, Canada (Tremblay et al. 1996*b*; Tremblay and Lucotte 1997). In these studies, insects from large-scale reservoirs had higher concentrations of MeHg than those from natural lakes.

Surma-Aho et al. (1986) collected Odonata and Trichoptera, which were analyzed as one group, whereas Tremblay et al. (1996*b*) and Tremblay and Lucotte (1997) analyzed individual taxa that were then grouped into FFGs similar to those used in this study. The results from Surma-Aho et al. (1986), Tremblay et al. (1996*b*), and our study can be compared by selecting data on Odonata and Trichoptera from both ELA and Québec, combining all data into either reservoir or natural lake categories, and converting all MeHg concentrations to nanograms per gram dry mass (using a conversion factor of dry mass/wet mass = 0.20). Comparisons of MeHg concentrations in natural lakes versus reservoirs were 51.6 versus 124.4, 62.3 versus 214.4, and 83.3 versus 179.3 ng·g d.m.$^{-1}$ in Finland, in Québec, and at the ELA, respectively. This comparison reveals that MeHg concentrations in Trichoptera and Odonata were about 2.5–3.0 times greater in reservoirs than in natural lakes. Only average means were presented in Tremblay and Lucotte (1997), and therefore, this study could not be included in the comparison.

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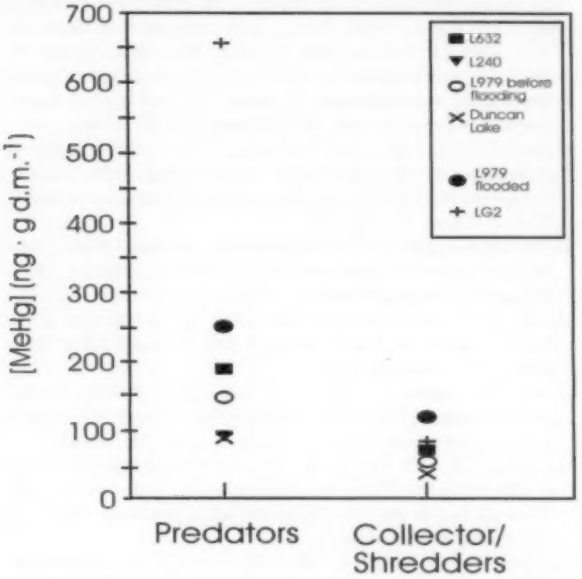
Fig. 4. MeHg concentrations in aquatic insects from the ELA flooding experiment (this study) and the Québec reservoir study (Tremblay et al. 1996*b*).



A more direct comparison can be made between the FFGs of Tremblay et al. (1996*b*) and this study. MeHg concentrations in collector/shredders from LG2, a 14-year-old Québec reservoir, and L979 did not vary markedly from natural Duncan Lake (Fig. 4). However, predators in the Québec reservoir averaged four times more MeHg than those in Duncan Lake. MeHg concentrations in predators from flooded L979 were lower than in the LG2 reservoir but higher than in Duncan Lake. Predaceous insects from Duncan Lake had similar MeHg concentrations to those in the ELA lakes used as references (L632, L240, and L979 before flooding; Fig. 4).

Functional feeding groups accumulated MeHg to different degrees in both the ELA and Québec studies. Predators from ELA reference lakes and from Duncan Lake in Québec had MeHg concentrations that were about two times higher than those in collector/shredders. Predators from LG2 and L979 had MeHg concentrations that were five and about three times greater, respectively, than in collector/shredders.

Bioaccumulation of MeHg in invertebrate food webs

MeHg concentrations increased significantly over time in both water and predaceous insects but not in collector/shredders. Peak concentrations of MeHg in water increased an average of 10 times in response to flooding (Kelly et al. 1997), but concentrations in predators exhibited a maximum threefold increase. Time lags may exist as MeHg moves through the aquatic food web. The experimental reservoir is very complex and has not reached a steady state; changes in the communities and environments of aquatic insects are far from complete. Moreover, the move towards a steady state was continually disrupted in the drawdown/reflooding cycle.

There were insufficient numbers of samples collected to make a definitive conclusion on MeHg increases in collector/shredders; however, our data are consistent with those from other studies. The lack of an obvious flooding response may have a number of explanations. First, herbivores may be inefficient in assimilating MeHg (i.e., MeHg may be less easily taken up from plant material, detritus, or water than from animal tissues). Second, some aquatic insects may be more efficient than others at depurating MeHg. Third, FFGs are exposed to different carbon sources and thus may experience different exposure to MeHg. Further research is needed on the relative importance of different routes of uptake and depuration of MeHg and on the importance of growth and metabolism in accumulation of MeHg by aquatic insects.

Other factors also may be important in interpreting our results. For example, Hg methylation is highest during warm months and under anoxic conditions (Watras et al. 1994). However, the examination of MeHg concentrations in aquatic insects from L632 did not reveal any seasonal trends. Life history data may have been useful in the interpretation of anomalous MeHg concentrations. The high concentrations in *L. americanus* and *Dytiscus* in both L979 and L632 in 1992 may have been related to the end of multiyear life cycles (Merritt and Cummins 1996) in these large predators. However, our study did not use a life history approach and these anomalous data remain unexplained.

Role of aquatic insects in bioaccumulation of MeHg by fish

The results of the Hg uptake experiment of Hall et al. (1997) and model simulations of Harris and Snodgrass (1993) and Rodgers (1994) indicate that most Hg in fish comes from the diet. Thus, increased MeHg concentrations in aquatic insects in response to reservoir flooding can explain elevated Hg concentrations in fish in these reservoirs. *Phoxinus neogaeus* were held in pens in L979 to quantify changes in rates of MeHg uptake in response to flooding. The diet of these fish, as determined by gut content analysis, was primarily aquatic insects (65–82%) and zooplankton (16–31%), with some algae and detrititus (0–11%) (R.A. Bodaly, Freshwater Institute, Winnipeg, Man., unpublished data). Average rates of MeHg uptake after flooding in 1993 and 1994 were 0.20 and 0.21 g $Hg \cdot m^{-2} \cdot year^{-1}$, respectively, compared with 0.08 and –0.02 g $Hg \cdot m^{-2} \cdot year^{-1}$ in the first two years prior to flooding (R.A. Bodaly, Freshwater Institute, Winnipeg, Man., personal communication), or a four to six times increase in net uptake of MeHg by fish in response to flooding.

Aquatic insects are part of a food web system that transfers MeHg from the physical environment to fish, and therefore, increases in MeHg concentrations in aquatic insects are an important consequence of reservoir creation. The results from this study will be used to calibrate a predictive MeHg model that will help evaluate the changes in Hg concentrations in fish in response to flooding (R. Harris, Tetra Tech Ltd., Oakville, Ont., personal communication). Further studies should explore the effects of life history and behaviour (e.g., life-span, metabolism and growth, habitat selection, and migration) on the bioaccumulation of MeHg in insects. The importance of different uptake routes of MeHg must also be established to help clarify how increases in MeHg concentrations in the physical environment of reservoirs lead

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to increased MeHg concentrations in insects and eventually fish.

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Quantitative genetic variation and genotype by environment interaction of embryo development rate in pink salmon (*Oncorhynchus gorbuscha*)

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Abstract: Quantitative genetic variation of development rate was evident among 20 half-sib and 40 full-sib families within each of two seasonally separate components of a population of pink salmon (*Oncorhynchus gorbuscha*) (H_o: no sire effect on temperature units at hatch, $P < 0.02$). Differences between averages of families spawned 3 weeks apart may have had genetic or environmental sources (e.g., in constant 8°C, early embryos hatched at 606 temperature units, and late embryos, at 625). Statistical interactions between paternal effects and environment (embryos were cultured in four temperature regimes, two simulated natural regimes and two constant temperatures; H_o: no sire by regime interaction effect on temperature units at hatch, $P < 0.09$) were weak evidence that genotype by environment interactions contributed to variation. Paternal effects in analysis of variance (evidence of additive genetic variation) were detected only at later stages. Evidences of genetic variation and of interactions between genotypes and environments are pertinent to resource conservation because they suggest that harvest management or hatchery practice have the potential to reduce genetic variation in salmon populations.

Résumé : Une variation génétique quantitative du taux de développement était apparente chez 20 familles de demi-frères et 40 familles de plein-frères au sein de deux composantes saisonnières distinctes d'une population de saumon rose, *Oncorhynchus gorbuscha*. (H_o : aucun effet du père sur le nombre d'unités de température au moment de l'éclosion, $P < 0{,}02$). Les écarts notés entre les moyennes des familles dont la fécondation présentait trois semaines d'intervalle peuvent s'expliquer par des facteurs génétiques ou environnementaux (p. ex. : à une température constante de 8°C l'éclosion des embryons se produisait à 606 unités de température pour les plus hâtifs et à 625 pour les plus tardifs). Les interactions statistiques entre les effets paternels et l'environnement (embryons élevés sous quatre régimes de température, deux régimes naturels simulés et deux à température constante; H_o : aucune interaction père-régime sur les unités de température à l'éclosion, $P < 0{,}09$) constituaient un indice faible du faible apport à la variation des effets de l'environnement sur le génotype. Une analyse de variance des effets paternels (évidence d'une variation génétique additive) n'a permis de mettre ces derniers en évidence qu'aux stades ultérieurs. L'existence de signes de variation génétique et d'interactions entre les génotypes et les environnements s'avère pertinente pour la conservation des ressources car elle indique que la gestion de la récolte ou les pratiques des piscicultures pourraient influer sur la variation génétique des populations de saumon.

[Traduit par la Rédaction]

Introduction

Stream temperature is associated not only with seasonal timing of spawning but also with embryo development rate in salmon populations; variations of rate among populations may have evolved as adaptations to different freshwater environments (Alderdice and Velsen 1978; Brannon 1987; Beacham and Murray 1987). For instance, fry of early-spawning pink salmon (*Oncorhynchus gorbuscha*) populations emerge later than fry of late-spawning fish when incubated at identical temperatures (Beacham and Murray 1987). Chum salmon (*O. keta*) fry of late-spawning fish emerge from gravel in a shorter time than those of early-spawning fish, despite their cooler environment and shorter incubation measured in degree-days (Beacham and Murray 1986). Variations of development rate within populations may also be important to fitness. Sources of variation of temperature-specific development rate, in addition to environmental differences, may include additive genetic, nonadditive genetic, and genotype by temperature interactions (Beacham 1988). To determine whether genetic variation underlies variation of development rate, we analyzed variation of development rate in families of Auke Creek pink salmon embryos in the laboratory.

Auke Creek is a small lake-fed creek located about 15 km north of Juneau, Alaska. A permanent weir, positioned just above extreme high tide, enables researchers to count individual immigrant adult salmon and to count individual

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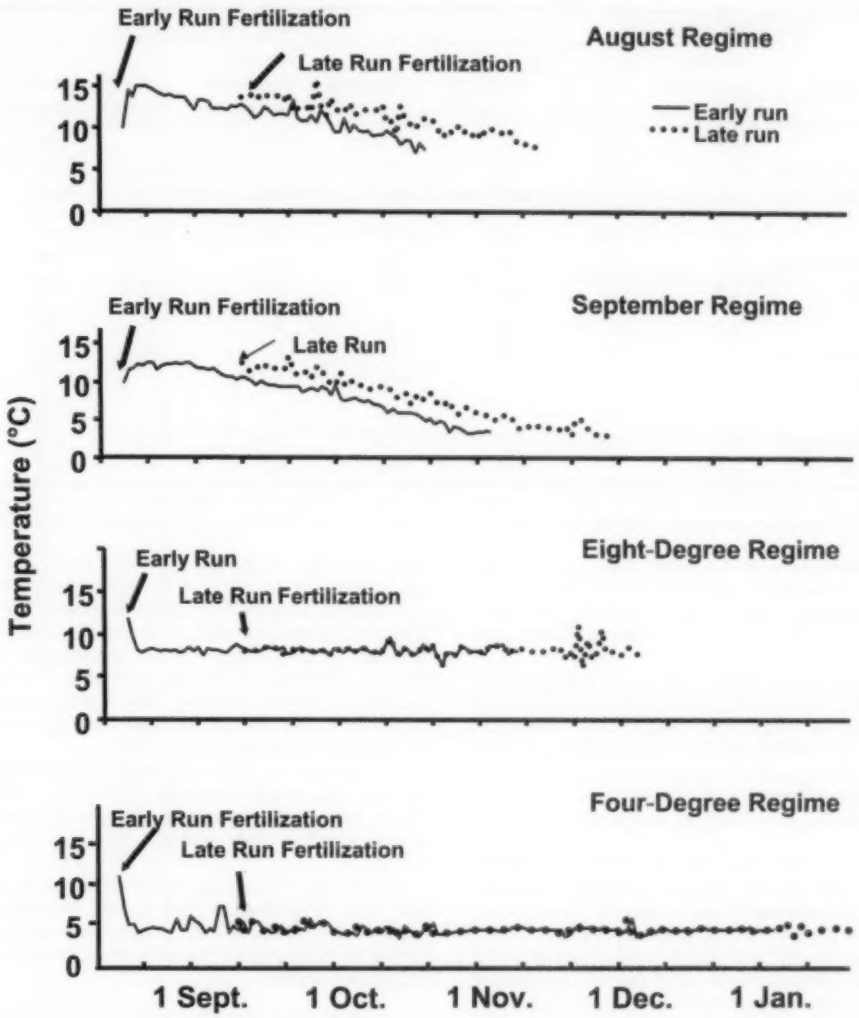
Fig. 1. Average daily temperatures during incubation of early- and late-run embryos in simulated natural regimes (declining temperature, modeled on average records of Auke Creek beginning in August and in September), and in 8 and 4°C (constant) incubation regimes.



emigrant juveniles. Pink salmon return to Auke Creek in two modes during August and September each year about 20 days apart (Taylor 1980; Gharrett and Smoker 1993*a*; Smoker et al. 1998; McGregor et al. 1998). Emigration of fry from late spawners at the end of embryonic development in the following spring is about 20 days after emigration of fry from early spawners, a difference detected by observation of genetically marked salmon (Lane et al. 1990; Gharrett and Smoker 1993*a*). The 20-day difference is maintained despite warmer temperatures experienced by early-run embryos in early development. The 20 days in autumn are as much as 10°C warmer than during the 20 days in spring, early fry accumulate as much as 200 more degree-days than late fry before emigration (Joyce 1986). This compensation may be an adaptive response to environmental variation related to additive genetic variation in Auke Creek pink salmon, or it may be a result of interactions between genotype and environment.

The purpose of this study was to estimate the additive genetic contribution to total variation in rate of development at three embryonic stages in Auke Creek pink salmon by partitioning phenotypic variation into its underlying genetic and environmental sources and to test for interactions of genotypes with their incubation environment.

Materials and methods

All pink salmon crossing the weir during the first week of the early-run return (mid-August) were held in submerged pens. On 14 August 1991, 40 ovulating females and 20 spermiating males were randomly sampled from about 400 fish. Eggs were kept over ice for 2 h prior to fertilization in the laboratory. We immersed fertil-

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Table 1. Average incubation temperature, accumulated temperature units (ATU, degree-days), predicted development rate (predicted numerical stage/ATU), and observed development rate (numerical stage/ATU) for families of early- and late-run Auke Creek pink salmon embryos incubated under several temperature regimes and observed at three stages of development.

		Early run				Late run			
Regime	Development stage	Average incubation temperature	ATU at sampling	Predicted stage/ATU	Observed stage/ATU	Average incubation temperature	ATU at sampling	Predicted stage/ATU	Observed stage/ATU
August	Epiboly	14.0	112	12	13	13.7	137	14	12
	Eye pigmentation	13.8	220	20	19	13.3	266	21	20
	Mid-hatch	12.1		662	664	11.6		662	655
September	Epiboly	11.9	130	13	12	11.9	142	14	13
	Eye pigmentation	12.0	215	20	20	11.5	230	20	23
	Mid-hatch	9.6		621	608	8.0		612	585
8°C	Epiboly	8.3	175	16	16	8.1	175	16	17
	Eye pigmentation	8.3	240	20	19	8.1	240	20	21
	Mid-hatch	8.1		612	606	8.1		612	625
4°C	Epiboly	4.7	220	18	20	4.1	263	20	21
	Eye pigmentation	4.5	283	19	22	4.1	317	21	23
	Mid-hatch	4.3		494	502	4.1		494	518

Note: Numerical stages are defined by Ballard (1973); stage predictions are by Jensen's (1988) model of pink salmon development time and are based on average incubation temperature at the time of sampling. Development rate at mid-hatch stage is given as accumulated temperature units.

ized eggs in 12°C stream water for 1 h before randomly assigning replicates of families to replicate compartments in incubators. Different incubation temperature regimes were established after cleavage (Fig. 1). The late run was similarly sampled on 9 September 1991.

A nested hierarchical mating design and mixed model analysis of variance were used to partition the variation in development rate into genetic and nongenetic components (Becker 1984). Mating 20 males (sires) with 40 females (dams) (two females per male) produced a total of 20 half-sib and 40 full-sib families from each of the two runs, a total of 80 families. Each full-sib family was divided into two replicate lots to provide two independent sets of records.

We incubated embryos under four different incubation temperature regimes (Fig. 1). Two regimes (August, September) simulated the Auke Creek stream temperatures encountered by early and late runs, the temperatures declined during early development. These regimes simulated the 10-year average of temperatures recorded for Auke Creek. Two constant regimes of 8 and 4°C represented natural average incubation temperatures during early stages and late stages of embryonic development respectively. Temperature control was provided by a system of chillers and heaters (Smoker 1992).

We sampled embryos at stage 14 (complete epiboly; Ballard 1973) and stage 20 (complete eye pigmentation); we used Jensen's (1988) prediction of temperature-days at these stages of development for pink salmon to schedule the samples (Table 1). Sample dates were determined from average temperatures in the incubators (Fig. 2), which were measured (±0.1°C) at least three times per day. Sums of daily average temperatures were used to determine sampling dates. Samples of 20 eggs from each family (10 from each family replicate) were preserved in 10% formalin. Daily observations of the proportion hatched in each replicate's incubator compartment provided two estimates of the median date of hatching for each family.

Rates of development were observed at epiboly, eye pigmentation, and midpoint of hatching. Rates were computed in each regime at each stage by dividing numerical embryo stages (Ballard 1973) by accumulated temperature units (degree-days) at observation. Comparison of 20 embryos from each family (10 from each replicate) to Ballard's (1973) descriptions of embryonic stages produced estimates of average embryo numerical stage in each sample. At epiboly we used the extent of advancement of the germ ring around the yolk to assign an embryo to one of five substages of development. At eye pigmentation we assigned embryos to stages according to a combination of caudal fin shape (straight or curved), presence and relative amount of caudal fin mesenchyme, and the ratio of longitudinal eye diameter to pectoral fin diameter (proximal to distal end).

We analyzed data after a variety of transformations to determine whether scale might artificially contribute to significance of any effects. Regardless of transformation, all analyses produced similar results; therefore, only analyses of untransformed data are reported.

We used an unbalanced, nested mixed-model analysis of variance with interaction to analyze rate of embryo development. Three models partitioned development rate into variation attributable to the main effects (run, sire, dam), or their interactions with temperature regime:

$$Y_{ijk} = \mu + S_i + D_{j(i)} + e_{ijk} \quad \text{(model 1)}$$

$$Y_{ijkl} = \mu + R_i + S_{j(i)} + D_{k(ij)} + e_{ijkl} \quad \text{(model 2)}$$

$$Y_{ijklm} = \mu + T_i + R_j + S_{k(j)} + D_{l(kj)} + T_i \times R_j + T_i \times S_{k(j)} + T_i \times D_{l(jk)} + e_{ijklm} \quad \text{(model 3)}$$

where Y is rate of embryo development rate (at epiboly, eye pigmentation, or mid-hatch) of an individual, μ is the population mean, R is the fixed effect due to run (early or late), S is the random effect due to sires nested within runs, D is the random effect due to dams nested within sires, T is the fixed effect due to incubation temperature regime, $T \times R$ is the effect due to regime by run interaction, $T \times S$ is the effect due to regime by sire interaction, $T \times D$ is the effect due to regime by dam interaction, and e is residual error.

Model 1 partitions variation in rate of embryo development into underlying effects for each run considered separately in each temperature regime. Model 2 partitions variation for runs combined for

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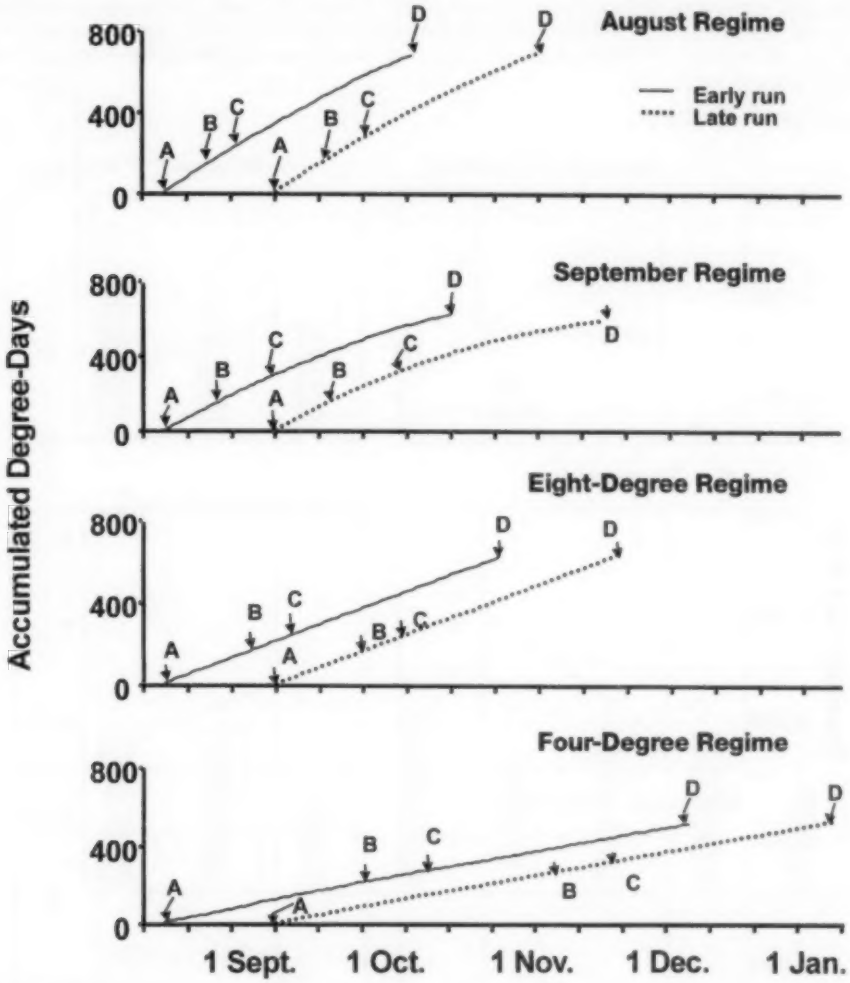
Fig. 2. Sampling schedule for early- and late-run pink salmon embryos in simulated natural (August, September) and in 8 and 4°C (constant) incubation regimes. Arrows indicate sampling times or important events: (A) fertilization; (B) epiboly; (C) eye pigmentation; (D) mid-hatch.



a temperature regime, and model 3 partitions variation for combined runs in all temperature regimes to estimate effects due to interactions of incubation regime (environment) and genotype (run, sire, dam). Run and temperature regimes were fixed effects, while sire and dam were random effects.

The general linear model (GLM) procedure of the Statistical Analysis System (SAS Institute Inc. 1990) type III sums of squares were used in the analysis. SAS also generated the expected mean squares used to construct significance tests, produced the coefficients used in variance component estimation, and computed the appropriate F tests. Details of the analyses are given in Hebert (1994) and Goddard (1995).

Total phenotypic variance (V_P) is composed of both genetic and environmental elements

$$V_P = V_A + V_N + V_E$$

where V_A is additive genetic variation, V_N is the combined variance due to dominance deviation and epistatic effects (nonenvironmental), and V_E is the variance due to environmental effects. Phenotypic variance is related to variance components, sire and dam effects, and the random error

$$V_P = \sigma_S^2 + \sigma_D^2 + \sigma_E^2.$$

Estimates of additive genetic variance within each run in each regime were obtained from the sire variance multiplied by four ($V_A = 4\sigma_S^2$; Becker 1984).

These variance components can be estimated from the mean squares (MS) derived from analysis of variance:

$$MS_{sires}\text{ estimates} \quad \sigma_E^2 + k_2\sigma_D^2 + k_3\sigma_S^2$$

$$MS_{dams}\text{ estimates} \quad \sigma_E^2 + k_1\sigma_D^2$$

$$MS_{error}\text{ estimates} \quad \sigma_E^2$$

where MS are the mean squares for each level of variation; σ_S^2, σ_D^2, and σ_E^2 are variances due to sires, dams, and error, respectively; and k_1, k_2, and k_3 are constants accounting for different

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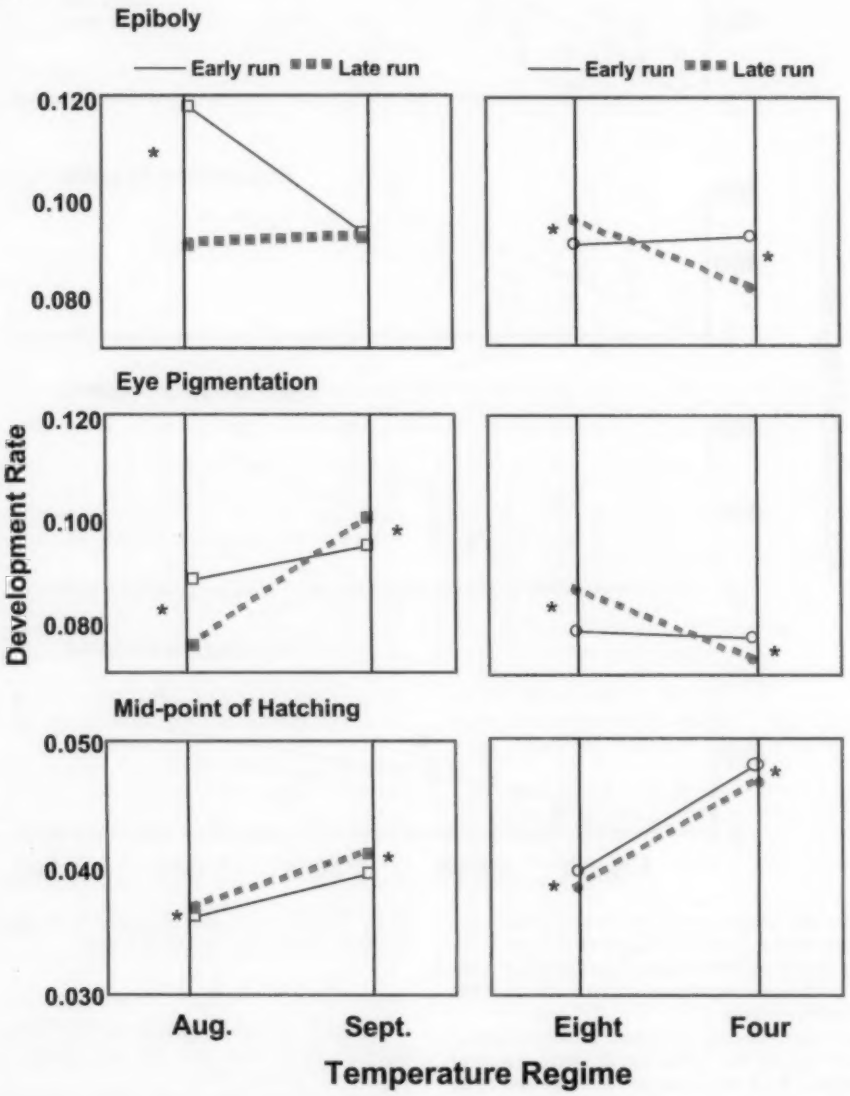
Fig. 3. Mean development rate of families of early- and of late-run pink salmon embryos at epiboly, eye pigmentation, and mid-hatch in simulated natural (August, September) and in 8 and 4°C (constant) incubation regimes. Early- and late-run observations in each regime are pooled and displayed pairwise (the two simulated natural regimes and the two constant regimes) showing effects due to regime, run, sire, dam, and interactions between regime and each of the effects in the model. Differences in development rate between early and late runs are all significant (*, $P < 0.0001$) except when measured at epiboly in the September regime ($P < 0.0737$). Interactions between runs and regimes are significant ($P < 0.0001$) at all stages of development except hatching in constant temperatures ($P < 0.1$).



numbers of progeny per family, adjusted to accommodate unbalanced data. Computational formulas for approximate standard errors for these variances were from Becker (1984).

Results

We considered temperature regime effects on development rate in the simulated natural and constant temperature regimes separately (Via 1984). The simulated natural regimes (i.e., August and September regimes; Fig. 1) were designed to give insight into variation in natural habitats, while the constant regimes (four degree and eight degree regime; Fig. 1) provided comparisons in constant environments. On average, early-run fish developed faster to epiboly and to eye pigmentation in the August regime than did the late-run fish but developed more slowly to eye pigmentation in the September regime (Fig. 3). The late-run fish hatched sooner (at fewer degree days) than did the early-run fish in both simulated natural regimes.

In the four-degree regime, the early-run fish developed

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Fig. 4. Phenotypic and additive genetic variation of early- (e) and late-run (l) embryo rate of development at epiboly, eye pigmentation, and mid-hatch in simulated natural (August, September) and in 8 and 4°C (constant) incubation regimes based on separate analysis of early- and late-run embryos. (*, significant sire effect at $P < 0.05$; error bars are SE estimated by the method of Becker 1984.) Interactions of sires and temperature regimes were each significant ($P < 0.05$) at each stage of development.

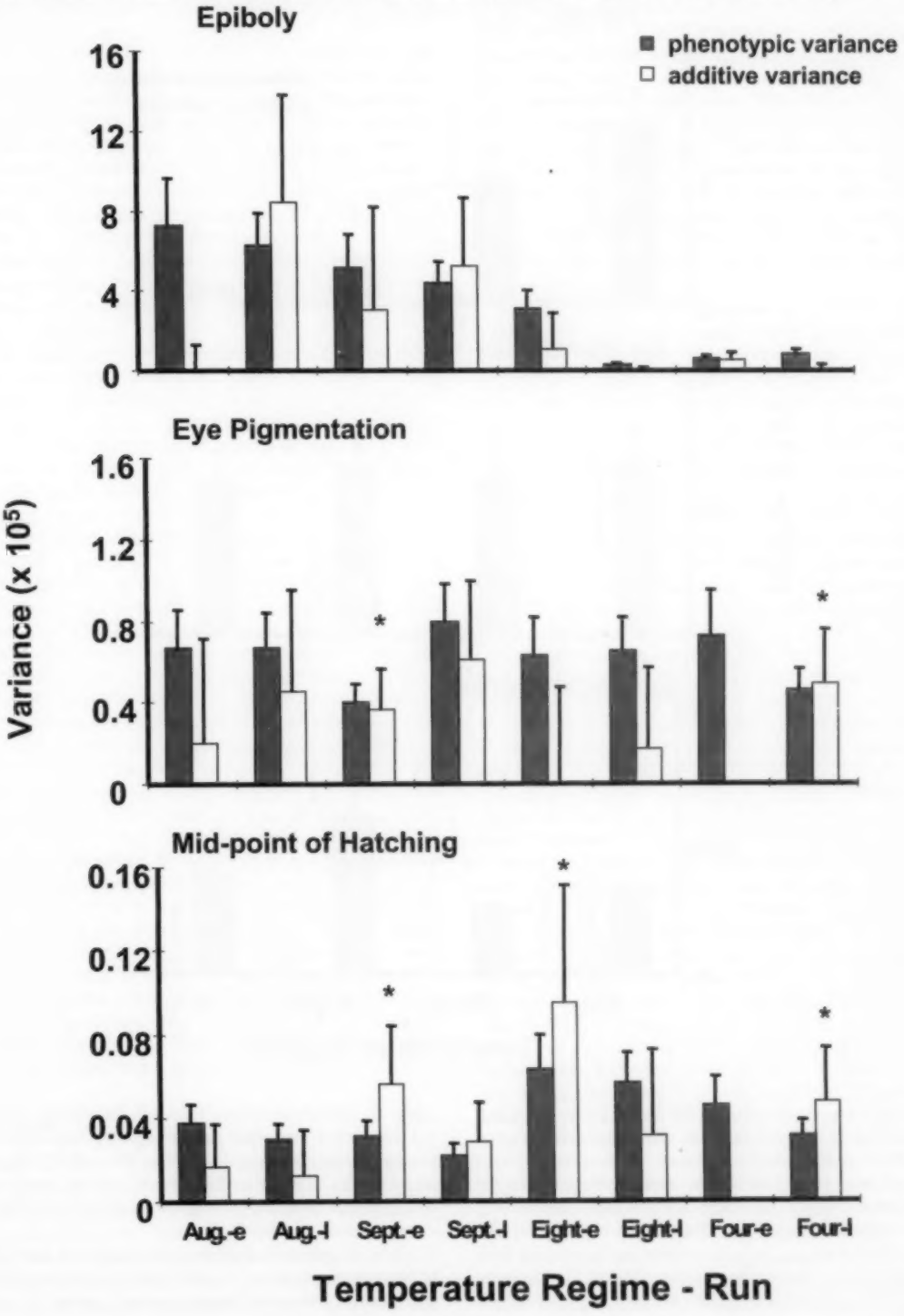

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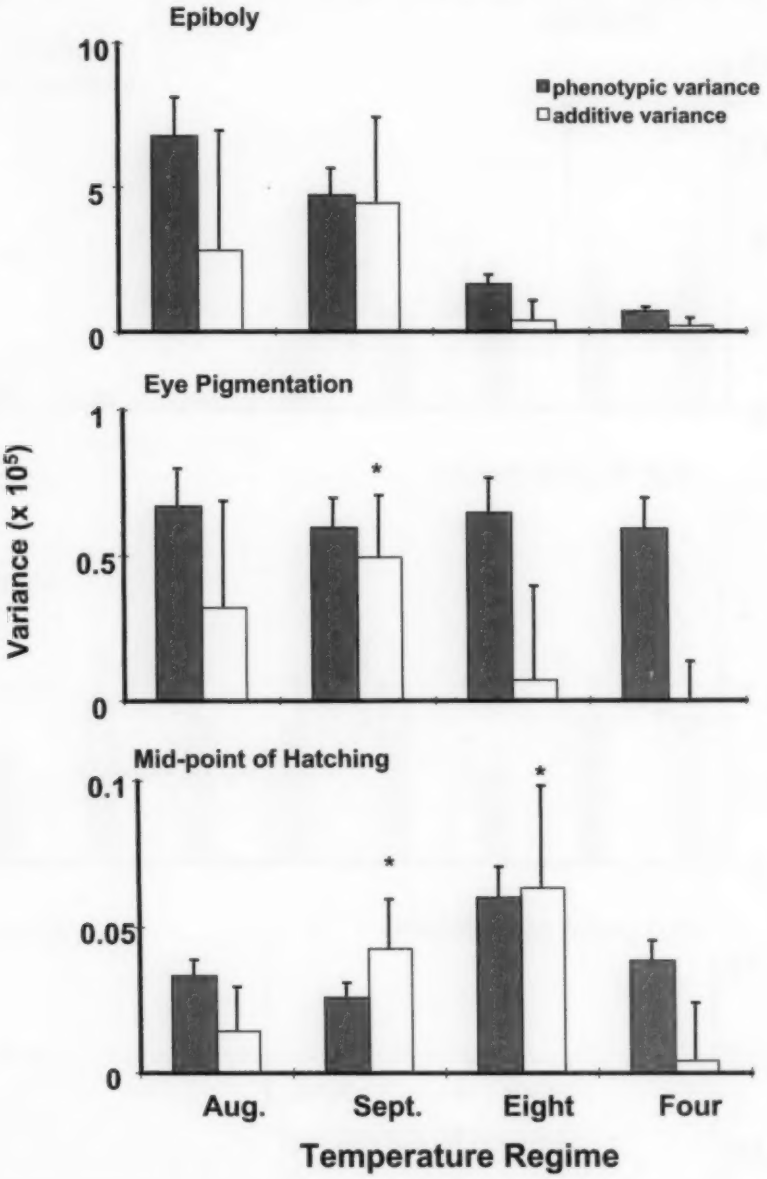
Fig. 5. Phenotypic and additive genetic variation of development rate measured at epiboly, eye pigmentation, and mid-hatch of pink salmon embryos incubated in simulated natural (August, September) and 8 and 4°C (constant temperature) regimes based on analysis of combined observation of early- and late-run embryos. *, significant sire effect at $P < 0.05$; error bars are SE estimated by the method of Becker 1984.



faster to every stage; whereas in the Eight Degree regime, the late-run fish developed faster to the prehatching stages, but the early-run fish hatched sooner. In the two regimes that were on average the coldest or warmer, the four degree and the August regimes, the early-run fish developed faster, with the exception of hatching in the August regime (Fig. 3).

The number of degree-days to mid-hatch decreased with the average temperature of the regime (Table 1). Embryos developing in the warmest regime (August) required an average of 660 degree-days to reach mid-hatch. Embryos in the September and eight degree regimes experienced similar average temperatures and required 596 and 616 degree-days, respectively, to reach mid-hatch; in contrast, embryos developing in the four degree regime required only 510 degree-days to reach mid-hatch.

Additive genetic variation of development rate (sire effect in separate analyses of model 1 for observations of the early and late runs) in each temperature regime was in no instance

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evident at epiboly but was evident ($P < 0.05$) in two of eight tests at eye-pigmentation stage (September regime for the early run and 4°C regime for the late run) and was evident in three of eight tests at hatching (Fig. 4). The simulated natural regimes caused more variation at epiboly than did the constant regimes.

Tests for additive genetic variation of development rate (sire effect in analyses of model 2 for observations of the combined runs in each temperature regime) demonstrate a pattern similar to the separate runs (Fig. 5). There is no evidence of sire effect at epiboly; only one of four tests shows a sire effect at eye pigmentation, but two of four tests shows a significant sire effect at hatching. This analysis considers that the early and late runs are components of one large population that are connected by (some) gene flow.

Both phenotypic and additive genetic variation apparently decreased approximately 10-fold as development progressed from epiboly to eye pigmentation and again from eye pigmentation to mid-hatch.

Tests for interactions of genotype and environment (sire by regime interaction effect in analysis of model 3) showed weak evidence of interactions in variation of development rate at epiboly ($P = 0.049$), eye pigmentation ($P = 0.034$), and hatching ($P = 0.085$). There were also dam by regime interactions for all three early-development traits except epiboly in the constant temperatures and eye pigmentation in the simulated natural regimes (all $P < 0.01$); however, this statistical interaction includes the effects of both additive and nonadditive genotype by temperature interactions, as well as any maternal effects (Becker 1984), effects that cannot be isolated in our experiment design. Interactions of run and regime contributed to variation of development rate measured at epiboly, eye pigmentation, and mid-hatch (all $P < 0.0001$ except hatch in constant temperatures $P < 0.1$). Embryos from neither of the runs were consistently faster or slower in development in the different temperature regimes; the interaction of runs and temperature regimes reflects this lack of consistency (Fig. 3).

Another way to test for the significance of the interaction between the genetic effects and the temperature is to combine all four regimes into a single analysis. This might not be considered appropriate, because two of the regimes simulate the natural environment while two are artificial; the advantage of this method is that it increases the power of the tests by increasing the degrees of freedom. Sire by regime interaction was significant for development at all three stages observed (all $P < 0.05$), and interactions between the environment with the effect of the dam and the effect of the run were also significant when all the regimes were considered together (all $P < 0.05$). These results are consistent with the results of the pairwise analysis.

Discussion

Our study produced three important findings: (1) rates of development were different in early- and late-run embryos, in ways generally explainable as adaptation to different environments; (2) sire effects were significant in several combinations of run and temperature regime at midpoint of hatch, which suggests that a proportion of variation in rate of development at hatch is due to additive genetic effects; and (3) variation of rate of development at all three observed stages of development may have been affected by interaction of sire and temperature regime.

Early- and late-run fish had different rates of development in different temperature regimes. One explanation for differences between the runs is that they are adapted to patterns of temperature change during early development determined by their return timing and that temperature cues are related to the progression of development. The early development (before eye pigmentation) of early-run embryos ordinarily occurs in warmer temperatures than that of late-run embryos. The stream temperatures encountered by the early-run fish are more variable from year to year than those encountered by late-run fish. Occasionally these early temperatures approach lethal limits. Additive genetic variation in the early run may ensure that some families survive extreme stream temperature variation.

Salmon populations that spawn at different times often produce fry with closely timed vernal entry to nursery environments, even when a late-returning population spawns in colder water than an early-returning population (Sheridan 1962; Brannon 1987). Different rates of embryonic development may be partly responsible for synchronization of pink salmon fry seaward migration with the annual spring bloom (Beacham and Murray 1988). Such different rates of development may be due to adaptations to specific temperature regimes. Adaptation to different temperatures may result in synchronization of emergent fry so that movement into the estuary occurs at a time that optimizes growth and survival. Historically, similar temporal spans have separated early- and late-run upstream and downstream migration in Auke Creek (Gharrett and Smoker 1993*b*). Because late-run incubation temperatures are lower than for the early run, late-run fish accumulate approximately 150 degree-days less than early fish upon fry emigration, both in the study by Joyce (1986) and in this study in which accumulated degree-days at hatching were about 660 in the warmest regime (August) and about 510 in the coolest regime (4°C). As a result duration of development is no longer for late fish than for early fish, which is consistent with a hypothesis that development rates in the two runs have adapted to different temperatures.

Other evidence supports the hypothesis. Variation between early and late fry migration into the estuary has been observed to be associated with variation of marine survival of Auke Creek pink salmon (Taylor 1980). Growth and survival of emigrant fry have been observed to peak and wane during the emigration period from Auke Creek in several seasons (S.G. Taylor, D.M. Mortensen, and A.C. Wertheimer, U.S. National Marine Fisheries Service (NMFS), Auke Bay Laboratory, Juneau, Aalska, personal communication). Their repeated observation of optimum emigration timing suggests that a stabilizing selection regime may have operated on development rate. However, stabilizing selection would be expected to reduce genetic variability (Scharloo 1964, but see Zhivotovsky and Feldman 1992), and we found evidence of additive genetic variation of development rate within runs. A plausible explanation of this contradiction is that it is possible that embryo development rate and seasonal timing of adult migration and spawning, both determinants of timing of fry emigration, are exposed to dispersive selection rather than stabilizing selection. Early-run

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fish with too late a run timing or too slow a development rate have an increased risk of mechanical disturbance during sensitive early development by the nest building of late-run fish (Smoker et al. 1998). This hypothetical source of dispersive selection acting on development rate would tend to increase the difference between runs, and we did find fixed differences of development rate between runs. The latter was possibly related to genetic differences. In the simulated natural temperature regimes, development of early-run fish was faster before epiboly than that of late-run fish; however, the late-run fish hatched after fewer degree-days.

We found evidence of additive genetic variability of development rate (significant sire effects in analysis of variance) only at later stages of development. This may have implications about genetic structure in populations of salmon. In theory, fitness as a trait should have little additive genetic variation (Falconer 1989; Price 1972). This expectation is often extended to fitness-related traits (those that do not have negative correlations with other fitness traits), that is, stabilizing selection usually produces an optimum mean phenotype in a population at equilibrium with its environment and removes additive genetic variation of the traits (Via and Lande 1985; but see Zhivotovsky and Feldman 1992). Our observation of additive genetic variation of development rate implies either that this event has little influence on fitness or that other factors maintain variation in the population. In fluctuating environments, genetic variability of life-history traits may be important for long-term fitness. Annual climate fluctuations especially influence the embryonic stages of a salmon's life history when risk of death is greatest, so genetic variation of development rate may be an important component of long term population fitness, thus explaining our observation.

That we observed significant sire variation (i.e., additive genetic variation) only at later stages of development is consistent with observations of development in other vertebrates in which the nuclear paternal genome does not contribute to embryo development until after epiboly (Balinsky 1976). Early development is pre-programmed by the maternal genome through gene products contained in the yolk. Consequently, genetic variation of early development is due only to maternal influences. As development progresses past epiboly and the embryonic nuclear genome (including paternal genes) is expressed, variation of development due to paternal genetic variation is possible.

There is evidence of genotype by environment interaction (Via 1984) in the lack of consistent relationship between rates of development in the runs in different regimes (Fig. 3) confirmed by significant sire by regime interaction effects in the analysis of variance. Genotype by environment interactions of fitness traits are well documented in domesticated animals and plants (Dickerson 1962; Bradshaw 1965; Via 1984) but have not been demonstrated in many natural populations. Genotype by environment interactions have also been reported for salmonid post-hatching development rate, growth rate, and survival (Ayles 1975; Klupp et al. 1978; Beacham and Murray 1988).

The interactions among genotypes and environments indicate variation in expression of the average genotype of a run in different temperature regimes. They suggest that each run is adapted to different environmental conditions and that some degree of phenotypic plasticity exists for all three traits (Via and Lande 1985).

We are surprised by a hundredfold decrease in total phenotypic variance from epiboly to hatching (Figs. 4 and 5). This observation has been repeated and verified in a similar independent study of an unrelated pink salmon population (even-year Auke Creek; Goddard 1995). We do not believe the decrease is an artifact of a declining relationship between observational error and development stage because hatching, the last stage we observed, was the least precisely determined stage. It may be that the declining variance is a reflection of developmental canalization.

Genetic variation of development rate means development rate is susceptible to selection. Evolutionary implications of variation of this life-history trait should be considered in management and culture of salmon; aquaculture managers and fisheries managers cannot ignore the genetic substructure observed in salmon populations. Careful managers must consider genetic structure and its effect on salmon life-history traits, such as embryonic rate of development, or else risk losing genetic variation. Losses of genetic variation would reduce not only the evolutionary fitness but also the harvestable productivity of populations.

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Low-effort regression estimation of daily ration in young walleye, *Stizostedion vitreum*

Sharook P. Madon

Abstract: I used a low-effort regression approach to estimate daily rations of pond-reared age 0 walleye, *Stizostedion vitreum* (16.0–37.2 mm total length). The regression model predicted average 24-h food mass ($\bar{F}_{24}$) from two or three consecutive point estimates of food mass (F_t) in fish guts taken at a sampling interval of 3 h. Predicted $\bar{F}_{24}$ values were multiplied by gastric evacuation rates and by 24 h to yield daily ration estimates. Accuracy tests of the low-effort regression model for age 0 walleye based on resampling revealed that 93–100% of predicted $\bar{F}_{24}$ values fell within 2 SEs of observed $\bar{F}_{24}$ values determined from nine fish collections over 24 h. The age 0 walleye regression model provided estimates of daily ration that were within the 95% confidence intervals around observed daily ration values for conspecifics in an independent test pond (this study) and in other distinct pond systems. The age 0 walleye regression model also provided estimates of daily ration that were within the 95% confidence intervals around five out of seven observed daily ration values for age 0 lake yellow perch, *Perca flavescens*, also a diurnal feeder like walleye. The model reliably provided estimates of daily ration for fish that were 1.5–4.5 times the mean wet weight of the largest fish included in the model's domain.

Résumé : J'ai utilisé une méthode de régression comportant peu de calculs pour estimer les rations quotidiennes de dorés jaunes (*Stizostedion vitreum)* d'âge 0 élevés en étangs (16,0 à 37,2 mm de longueur totale). Le modèle de régression a permis de calculer la quantité de nourriture ($\bar{F}_{24}$) moyenne sur 24 h à partir de deux ou trois estimations ponctuelles consécutives de la quantité de nourriture (F_t) présente dans les viscères des poissons, prélevée à intervalle de 3 h. Les valeurs $\bar{F}_{24}$ prévues ont été multipliées par le taux d'élimination gastrique et par 24 h pour obtenir des estimations de la ration quotidienne. Les tests d'exactitude de ce modèle de régression pour les dorés jaunes d'âge 0 fondés sur le rééchantillonnage ont montré que de 93 à 100 % des valeurs F_{24} prévues se trouvaient à moins de 2 écarts-types des valeurs $\bar{F}_{24}$ observées, calculées à partir de neuf collectes de poissons sur une période de 24 h. Le modèle de régression pour les poissons d'âge 0 a permis d'obtenir des estimations de la ration quotidienne situées dans l'intervalle de confiance de 95 % autour des valeurs observées de la ration quotidienne pour des congénères dans un étang d'essai indépendant (la présente étude) et dans d'autres systèmes d'étangs distincts. Le modèle a aussi permis d'obtenir des estimations de la ration quotidienne situées dans l'intervalle de confiance à 95 % pour environ 5 des 7 valeurs observées de la ration quotidienne pour la perchaude d'âge 0, poisson à alimentation diurne comme le doré. Le modèle a fourni avec fiabilité des estimations de la ration quotidienne des poissons correspondant à 1,5 à 4,5 fois le poids frais moyen du plus gros poisson inclus dans le domaine du modèle.

[Traduit par la Rédaction]

Introduction

Estimates of fish daily ration are usually derived directly by measuring food mass in guts over 24-h periods (Eggers 1977; Elliott and Persson 1978; Boisclair and Leggett 1988) or indirectly via bioenergetics models (Lyons and Magnuson 1987; Hewett and Stewart 1989). Direct methods are labor intensive, requiring 24-h association with sampling sites at the minimum for a single daily ration estimate. Consequently, the spatial and temporal resolutions of such food consumption studies are often very limited (Nakashima and Leggett 1978; Boisclair and Leggett 1988; Ney 1990; Hayward et al. 1991). Bioenergetics models predict consumption when temperature and growth are known (Ney 1990, 1993). These models involve from 15 to 30 parameters to describe temperature and weight dependencies of consumption, metabolism, and waste losses. Error is associated with each parameter (Bartell et al. 1986), and the accuracy of consumption estimates derived via bioenergetics models has often been questioned (Minton and McLean 1982; Boisclair and Leggett 1989; Wahl and Stein 1991; Ney 1993). Also, because of interspecific and ontogenetic differences, parameters of bioenergetics models should be determined separately for each distinct species/life stage combination (Post 1990; Hewett and Johnson 1992; Madon and Culver 1993). Model parameter borrowing is common, however, across species and life stages and is likely a source of substantial error in bioenergetics model estimates (Ney 1993). In light of the limitations associated with existing methods for estimating food consumption of fishes, more accurate and less labor-intensive approaches are highly desired (Ney 1990).

A low-effort approach was developed by Hayward et al. (1991) to estimate daily ration of juvenile and adult yellow

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Table 1. Wet weights (mg) (SE) of age 0 walleye during May–June 1990 in model ponds and an independent test pond.

Date	Model ponds		Independent test pond (low density)
	Low density	High density	
May 7	—	—	17.6 (0.5)
May 10	35.7 (0.9)	33.3 (0.6)	31.3 (0.8)
May 14	54.9 (1.2)	47.2 (0.6)	45.2 (1.1)
May 17	76.8 (2.2)	62.9 (1.2)	63.8 (1.6)
May 21	124.1 (2.5)	94.0 (2.2)	101.2 (2.5)
May 24	165.3 (3.8)	132.7 (2.2)	141.5 (4.1)
May 31	287.0 (4.5)	229.1 (5.3)	286.7 (10.8)
June 4	350.6 (5.1)	283.3 (6.0)	—

Note: Walleye were collected from May 10 to June 4 from one low-density (25 fish·m^{-3}) and one high-density (50 fish·m^{-3}) model pond to generate regressions between average daily food weight ($\bar{F}_{24}$) and time-specific point estimates of food weight (F_t) in their guts for the multiperiod model. The $\bar{F}_{24}$ and F_t were also determined for walleye collected from an independent test pond (25 fish·m^{-3}) between May 7 and 31, 1990. The data generated from the test pond were used for corroborating the multiperiod model.

perch, *Perca flavescens*, in Lake Erie. This approach was later applied to a reservoir population of larval gizzard shad, *Dorosoma cepedianum* (Hayward and Hiebert 1993). The low-effort approach is based on the premise that across 24-h periods, fixed relationships exist between food weights in a fish population's guts at certain times of day (F_t) and the 24-h average food mass ($\bar{F}_{24}$) (see Hayward and Hiebert 1993 for a description of the theoretical basis behind the model). If strong correlations exist between the F_t values and $\bar{F}_{24}$ values in three or four consecutive 3-h time periods over several 24-h feeding periods, previous findings suggest that a multiperiod regression model can be developed to accurately estimate $\bar{F}_{24}$ values from just three or four food mass samples collected within a 6- to 9-h period (Hayward and Hiebert 1993). Model predictions of $\bar{F}_{24}$ are multiplied by the gastric evacuation rate and then by 24 h to yield daily ration (Eggers 1977; Hayward and Hiebert 1993).

The low-effort regression approach is a promising way to reduce sampling effort in direct assessments of fish daily ration and to permit increased spatial coverage in such studies. Studies to date have not tested the ability of such models to provide accurate estimates of daily ration of conspecifics in distinct systems, such as those separated in both space and time (Hayward et al. 1991; Hayward and Hiebert 1993). Furthermore, because this approach relies on fundamental relationships between F_t values and $\bar{F}_{24}$ values, a model developed for one species should be applicable to other species with similar feeding patterns (e.g., diurnal feeders). Therefore, this approach needs to be tested further with other fish species and determinations made as to the extent that any single model can be applied to distinct populations of conspecifics (Hayward and Hiebert 1993). Hayward and Hiebert (1993) suggested analyses of existing 24-h feeding data sets as one way to expedite development and testing of low-effort regression models for other fish species.

I used Hayward and Hiebert's (1993) approach to develop and test a multiperiod regression model for estimating daily ration of pond-reared age 0 walleye, *Stizostedion vitreum*. For this, I used existing data from a series of 24-h feeding studies that were collected as part of a larger study on walleye energetics (Madon and Culver 1993). I used diel feeding data collected from two ponds on seven separate dates during May–June 1990 to develop a multiperiod regression model. Similar data collected from a third pond were used to test the regression model's ability to estimate $\bar{F}_{24}$ values and daily ration of age 0 walleye in an independent pond. I also tested for the model's ability to provide daily ration estimates for (*i*) age 0 walleye in geographically and temporally distinct pond systems (Fox 1991) and (*ii*) age 0 yellow perch, also diurnal feeders like walleye, in Lake St. George (Post 1990).

Materials and methods

Field and laboratory procedures

I used 24-h feeding data derived from experiments conducted in ponds at the St. Mary's Fish Hatchery, St. Mary's, Ohio, as part of a study on the energetics of larval and juvenile walleye (Madon and Culver 1993). Ponds were filled with water from nearby Grand Lake and therefore contained natural assemblages of zooplankton. Details on pond filling, stocking, and diel sampling of fish for analysis of food mass in guts are explained elsewhere (Madon 1993; Madon and Culver 1993).

Nine collections of age 0 walleye were made at 3-h intervals over 24-h periods (09:00–09:00 or 12:00–12:00) from three ponds between May 7 and June 4, 1990 (Table 1). The 3-h time periods began and ended at about 09:00, 12:00, 15:00, 18:00, 21:00, 24:00, 03:00, and 06:00. For each sampling date, the nine samples of gut contents of walleye collected from one low-density (25 fish·m^{-3}) and one high-density (50 fish·m^{-3}) pond were weighed (methodology described in Madon and Culver 1993) to generate 3-h-apart point estimates of food mass in guts (F_t) and average 24-h food weights ($\bar{F}_{24}$). Each F_t estimate was determined as the mean of weight-specific food weights (grams of food per gram of fish, wet mass basis) in guts of five walleye. True mean 24-h food weights in walleye guts were assumed to be accurately reflected by the average of the nine food mass point estimates (Elliott and Persson 1978; Hayward and Hiebert 1993). Data on $\bar{F}_{24}$ and F_t collected from these two ponds, henceforth called model ponds (Table 1), were used to generate regressions for the multiperiod model. Data on $\bar{F}_{24}$ and F_t were similarly estimated for walleye collected from a third low-density (25 fish·m^{-3}) pond, henceforth called the test pond (Table 1). The ability of a multiperiod regression model developed from the two model pond's data sets to estimate $\bar{F}_{24}$ values in the test pond was tested.

Multiperiod model development

I used procedures described by Hayward and Hiebert (1993) to develop a multiperiod regression model for age 0 walleye. First, over all sampling dates for each 3-h time period, I examined linear regressions of $\bar{F}_{24}$ on F_t. Next, I constructed a single multiperiod regression model that estimated $\bar{F}_{24}$ values from food mass data collected from up to three 3-h-apart time periods. Criteria for choosing these 3-h time periods were that (*i*) they were consecutive in time (to minimize time in the field) and (*ii*) the associated regressions had relatively high r^2 values. Data of $\bar{F}_{24}$ and F_t from the selected time periods were used to construct the multiperiod regression model using a stepwise procedure with backward selection, accompanied by residual analysis to select independent variables (Hayward and Hiebert 1993). The multiperiod model was used to estimate $\bar{F}_{24}$ by incorporating F_t values from any one, two, or up to three consecutive 3-h time periods included in the model. When point estimates of F_t from more than one time period were used, $\bar{F}_{24}$ values were generated separately for each time period and then averaged.

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Multiperiod model internal validation and corroboration

I used two tests, the first to conduct an internal validation of the model and the second to corroborate estimates of $\bar{F}_{24}$ values made with the multiperiod regression model. The first test involved an internal test of the model within the regression's domain (model ponds only) using a jackknife procedure (described in Hayward and Hiebert 1993). In this procedure, I reconfigured the multiperiod model by removing all $\bar{F}_{24}$ and F_t data from one 24-h sampling date (1 sampling date × 2 model ponds × 3 time periods = 6 pairs of data points removed for each reconfiguration). In this manner, I generated seven reconfigured multiperiod models, one for each sampling date for which data were removed. I used each multiperiod model with reconfigured coefficients to estimate $\bar{F}_{24}$ for the particular sampling date for which data were removed. This method allows for independent comparisons between estimated and observed values of $\bar{F}_{24}$. I evaluated model accuracy by estimating the standardized error (SE) associated with each predicted $\bar{F}_{24}$ as

$$\frac{\text{Estimated } \bar{F}_{24} \text{ value} - \text{observed } \bar{F}_{24} \text{ value}}{\text{SE of observed } \bar{F}_{24} \text{ value}}$$

and generating pooled error distributions across all sampling dates. I generated separate error distributions of model estimates when F_t values from one, two, and three time periods contributed information to the multiperiod regression model and considered the model's performance to be adequate if at least 90% of all $\bar{F}_{24}$ predictions fell within 2 SEs (about 95% confidence interval (CI)) of observed $\bar{F}_{24}$ (Hayward and Hiebert 1993).

The second test involved comparisons of multiperiod model predictions of $\bar{F}_{24}$ values with observed $\bar{F}_{24}$ values for (*i*) age 0 walleye in the independent test pond (this study), (*ii*) age 0 walleye in four distinct ponds separated in space and time (Fox 1991), and (*iii*) age 0 lake yellow perch (Post 1990). This procedure evaluated the ability of the regression model developed from the model ponds data sets to predict $\bar{F}_{24}$ values for walleye and yellow perch in truly distinct systems, when F_t data from one to three 3-h time periods collected from those systems were entered into the model. I conducted two independent statistical evaluations that tested the degree to which predicted values of $\bar{F}_{24}$ from the multiperiod regression model matched observed values (see Rice and Cochran 1984 for details of these tests). (*i*) A mean square error (MSE) was generated via regressions of predicted on observed values. The MSE was then partitioned into the mean and slope (systematic errors) and residual (random error) components. Systematic error proportions close to 0 and random error proportions close to 1 indicate that the majority of errors are random and not due to the mean and slope components. (*ii*) Bonferroni joint confidence intervals test the null hypothesis that the intercept and slope of the regressions of predicted versus observed values of $\bar{F}_{24}$ are 0 and 1, respectively. Tests that support the null hypothesis indicate good agreement between predicted and observed values. Because errors are associated with both predicted and observed values of $\bar{F}_{24}$, I used the geometric mean regression approach (model II regression), instead of standard regression, to estimate the intercepts and slopes of regressions of predicted on observed values (Sokal and Rohlf 1981; Krebs 1989).

Walleye daily ration

I used the Elliott and Persson (1978) method (E-P model) to estimate daily ration (grams of food consumed per gram wet mass of fish per day) of walleye in the test pond for each sampling date. The E-P procedure is a widely used method that provides standard estimates of daily ration against which other estimates of food consumption may be compared (Cochran 1979; Boisclair and Leggett 1988; Hayward et al. 1991). The E-P method requires information on gut evacuation rates and food mass point estimates over 24 h to estimate daily ration. Temperature- and weight-specific gut evacuation rates were predicted from an empirically derived model for age 0 walleye (Madon and Culver 1993). Gut evacuation rates were combined with point estimates of food mass in walleye guts determined at 3-h intervals over 24 h to yield E-P daily ration estimates for each sampling date. I calculated proportional errors that represented 95% CIs around each E-P daily ration estimate (Zar 1974; Sokal and Rohlf 1981; Post 1990) as

$$\text{SS} = \text{SE}^2 n(n-1)$$

where SS and SE are the sum of squares and standard errors, respectively, of each 3-h point estimate of food mass (grams of food per gram of fish, wet mass basis) in guts of walleye and n is the number of walleye that were examined to estimate food weights. Next, I calculated the pooled variance (PV) for each daily ration estimate:

$$\text{PV} = \sum_{i=1}^{9} \text{SS} \Big/ \sum_{i=1}^{9} (n-1)$$

where i = 1–9 indicates the nine time periods for which point estimates of food mass were generated within each 24-h period. The pooled standard error (PSE) around each daily ration estimate was generated next:

$$\text{PSE} = (\text{PV}/N)^{1/2}$$

where N is the total number of fish analyzed during a 24-h period for food mass estimates. The proportional error (PE) was estimated as

$$\text{PE} = (\text{PSE} \cdot t\text{-score}) / \bar{F}_{24}$$

where t-score is $t_{(1-\alpha,\ n-1)} = t_{(1-0.05,\ 8)} = 2.31$ and $\bar{F}_{24}$ is the average 24-h food mass (derived from nine point estimates of food weight).

For each sampling date, I used the multiperiod regression model to predict $\bar{F}_{24}$ values from one to three point estimates of food mass in the test pond. Daily ration values were estimated by multiplying predicted $\bar{F}_{24}$ by evacuation rates and then by 24 h (Eggers 1977). I also tested for the ability of the model to provide reliable estimates of daily ration in walleye conspecifics in truly distinct systems and in other species with similar feeding patterns. For this, I used the multiperiod regression model to predict $\bar{F}_{24}$ values for age 0 walleye in four ponds that were geographically and temporally distinct from ponds in this study (Fox 1991) and age 0 yellow perch on seven separate dates in Lake St. George, Canada (Post 1990). Age 0 yellow perch, like age 0 walleye, show diurnal feeding patterns (Post 1990). For predicting $\bar{F}_{24}$ values, I entered F_t data reported in Fox (1991) and Post (1990) from around 15:00, 18:00, and 21:00 into the multiperiod regression model for each pond, or date, respectively. Daily ration values were estimated by multiplying predicted $\bar{F}_{24}$ values by evacuation rates reported by Fox (1991) and Post (1990) and then by 24 h. I generated 95% CIs around observed daily ration values for age 0 walleye using the standard errors reported around each 3-h point estimate of food mass (Fox 1991) and the proportional error method described above. I used the observed daily ration values ± 95% CIs reported by Post (1990) for age 0 yellow perch. Post (1990) used the same proportional error method reported in this study to estimate his 95% CIs. Estimated daily ration values were considered to be corroborated if they fell within the 95% CIs of corresponding observed daily ration values. The 95% CIs around the observed daily ration values were conservative, as they did not include errors associated with the evacuation rate estimates. These conservative CIs provide for a more rigorous test of the model.

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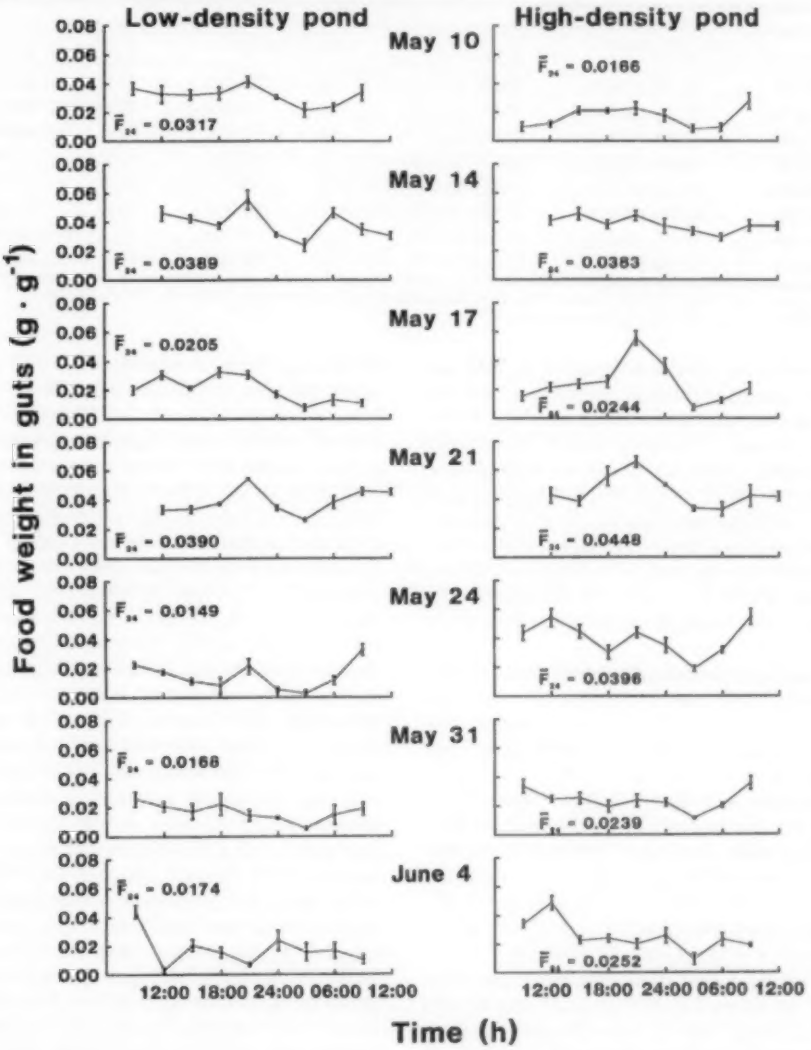
Fig. 1. Diel variation of food weights (± SE) in guts of age 0 walleye in two model ponds. Across all dates, for each of the 3-h time periods, point estimates of food weight (F_t, n = 5 fish for each point estimate) were regressed with corresponding values of average daily food weights ($\bar{F}_{24}$). Eight such regressions of $\bar{F}_{24}$ on F_t were determined.



Results and discussion

Walleye diel feeding and multiperiod model

The amplitude of point estimates of food weights (F_t) in walleye guts ranged widely between test ponds and sampling dates (Fig. 1) due to differences in food availability (two-way ANOVA with date and site as categories: n = 9 time periods × 7 dates × 2 sites = 126; date: $F_{6,112}$ = 15.2, $p < 0.0001$; site: $F_{1,112}$ = 8.0, p = 0.005; Fig. 1). Hayward and Hiebert (1993) indicated that strong correlations between $\bar{F}_{24}$ and F_t can be expected in fishes that show fairly consistent 24-h feeding patterns across days and that correlations would be insignificant during periods when feeding ceases. However, a significant interaction term for F_t values across dates and test ponds (date × site: $F_{6,112}$ = 7.0, $p < 0.0001$) revealed that the feeding cycles were relatively unstable, indicating that this method might be relatively robust against violation of the assumption of a stable feeding cycle. Correlations between $\bar{F}_{24}$ and F_t for age 0 walleye were significant for all 3-h time periods (Table 2), although the strongest correlations ($r^2 > 0.70$) were for time periods between 15:00 and 06:00. Unlike larval gizzard shad, which fed during daylight but stopped feeding at night (Hayward and Hiebert 1993), walleye in this study on several occasions fed during the night (95% CIs around F_t values for 24:00 and 03:00 rarely included 0). Highly significant correlations between 24:00 and 03:00 were observed in this study (Table 2) because walleye often fed during the night.

Strong correlations of $\bar{F}_{24}$ and F_t between 15:00 and 06:00 indicated that, according to selection criteria stated earlier,

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Table 2. Linear regressions ($\bar{F}_{24} = \alpha + \beta F_t$) relating time-specific point estimates of food weight (F_t, g·g^{-1}) to estimates of average 24-h food weights ($\bar{F}_{24}$) in guts of age 0 walleye collected from one high-density (50 fish·m^{-3}) and one low-density (25 fish·m^{-3}) fish pond.

Time period	Model coefficient		Regression statistic			
	α (SE)	β (SE)	N	r^2	F	p
09:00	0.010 (0.005)	0.594 (0.153)	14	0.556	15.0	0.002
12:00	0.011 (0.004)	0.551 (0.124)	14	0.620	19.6	0.001
15:00*	0.002 (0.003)	0.890 (0.099)	14	0.871	81.0	<0.001
18:00*	0.006 (0.004)	0.758 (0.129)	14	0.743	34.7	<0.001
21:00*	0.011 (0.004)	0.483 (0.090)	14	0.707	29.0	<0.001
24:00	0.007 (0.004)	0.780 (0.126)	14	0.762	38.4	<0.001
03:00	0.013 (0.003)	0.900 (0.132)	14	0.794	46.4	<0.001
06:00	0.009 (0.003)	0.815 (0.123)	14	0.787	44.3	<0.001

Note: An asterix indicates each of the three consecutive time periods from which $\bar{F}_{24}$ and F_t data were chosen to construct the multiperiod model.

$\bar{F}_{24}$ could be estimated from a point estimate of F_t from any one of these six time periods (Table 2). However, I included $\bar{F}_{24}$ and F_t data only from daylight periods of 15:00, 18:00, and 21:00 with correlations (r^2) ranging from 0.707 to 0.871 in the regression model (Table 2). These daylight periods were preferred over the 24:00, 03:00, and 06:00 periods on the assumption that daytime sampling would be logistically more desirable in most cases than sampling at night. However, strong correlations of $\bar{F}_{24}$ and F_t at 24:00, 03:00, and 06:00 (Table 2) indicated that a regression model based on these periods could be developed if sampling at night is ever necessitated.

The multiperiod regression model developed over the daytime periods of 15:00, 18:00, and 21:00 was

$$\hat{\bar{F}}_{24} = 0.006 + 0.765F_t + 10.333F_t^2 - 0.608F_t^2t$$

where $\hat{\bar{F}}_{24}$ is the regression estimate of $\bar{F}_{24}$, F_t is as previously defined, and t is the time of day (in 24-h time) that a point estimate of food mass in walleye guts was made; $r^2 = 0.812$, $F_{(3, 38)} = 53.1$, $p < 0.0001$, $N = 42$.

Multiperiod model internal validation and corroboration

The multiperiod model shown above was reconfigured for each sampling date by removing all $\bar{F}_{24}$ and F_t data in both model ponds for that date. The $\bar{F}_{24}$ was then predicted for the date for which data were removed using F_t from one up to three time periods. This procedure provided for an internal validation of the model. Error distributions were normal (Shapiro–Wilk test for $N < 50$, $p > 0.05$) and indicated no bias tendency in regression predictions of $\bar{F}_{24}$ (t-test, $p > 0.05$). Accuracy of $\bar{F}_{24}$ values predicted by the multiperiod regression model increased as the number of time periods providing food mass data to the model (one to three) increased. However, the model met the accuracy criterion (>90% of predicted $\bar{F}_{24}$ values must lie within 2 SEs of observed $\bar{F}_{24}$ values) even when food mass data from only one time period were included. When F_t data from any one to three time periods were included, from 93 to 100% of $\bar{F}_{24}$ values predicted by the model fell within 2 SEs of observed values. These results indicate that the multiperiod regression model predicted $\bar{F}_{24}$ accurately by including food mass data determined from a minimum of only one fish collection made at either 15:00, 18:00, or 21:00. These results differ from those of Hayward and Hiebert (1993), who reported that the performance of their multiperiod model for larval gizzard shad met a similar accuracy criterion only when F_t data from three or four time periods were included in the model. Therefore, this low-effort regression approach seems even more robust when applied to age 0 walleye, despite the fact that the gizzard shad regression model was considerably stronger than the one developed for age 0 walleye in this study ($r^2 = 0.91$ for larval gizzard shad model versus $r^2 = 0.812$ in this study). Conversely, these differences could merely be due to differences in SEs of $\bar{F}_{24}$ values determined in this study and that of Hayward and Hiebert (1993). If lower SEs are observed in Hayward and Hiebert's (1993) study, then these authors are more likely to predict $\bar{F}_{24}$ values that will fall outside the 2 SE range.

I used the above multiperiod regression model derived from walleye food mass data in the two model ponds (one low and one high fish density pond) to predict $\bar{F}_{24}$ values for walleye in the separate test pond (low fish density). I specifically chose data from one low fish density and one high fish density pond for model development to include a wider range of F_t and corresponding $\bar{F}_{24}$ values due to differences in food availability and consequently food consumption in ponds stocked with different fish densities (Madon 1993). A model thus developed with data from ponds with different fish densities is likely more robust than one developed with data from ponds stocked with the same fish density. Furthermore, walleye in the test pond showed significantly higher food consumption as a result of increased food availability due to lower fish survival (Madon 1993; Madon and Culver 1993) than walleye in the model ponds. Therefore, the test pond served as an independent system for regression model corroboration. The $\bar{F}_{24}$ values predicted by the model when F_t data from one to three time periods were included were corroborated by observed values of $\bar{F}_{24}$ using partitioning of mean square error (MSE) and Bonferroni joint confidence intervals tests (Table 3). These procedures tested for the model's ability to accurately predict $\bar{F}_{24}$ for a walleye population in a distinct environment. Partitioning of MSE for all combinations of time periods revealed that deviations of predicted versus observed values of $\bar{F}_{24}$ were largely due to ran-

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Table 3. Partitioning of MSE into systematic (mean and slope) and random (residual) proportions for relationships between average daily food weight ($\bar{F}_{24}$) observed in the independent test pond (this study), age 0 walleye raised in four ponds (Fox 1991), and age 0 yellow perch in Lake St. George, Canada (Post 1990), and $\bar{F}_{24}$ predicted from the multiperiod model for age 0 walleye.

Predicted $\bar{F}_{24}$ using F_t from:	Source of error			Bonferroni joint CI	
	Mean	Slope	Residual	$\beta_0 \pm$ 95% CI	$\beta_1 \pm$ 95% CI
Independent test pond					
One time period					
15:00	0.006	0.001	0.993	0.005±0.007	0.879±2.047
18:00	0.003	0.001	0.996	0.004±0.009	0.826±0.302
21:00	0.012	0.021	0.967	0.001±0.012	1.026±0.404
Two time periods					
15:00 and 18:00	0.000	0.013	0.987	0.006±0.005	0.801±0.169
15:00 and 21:00	0.008	0.002	0.990	0.005±0.006	0.885±0.204
18:00 and 21:00	0.001	0.000	0.999	0.004±0.009	0.880±0.287
Three time periods					
15:00, 18:00, and 21:00	0.002	0.007	0.991	0.005±0.008	0.839±0.200
Distinct age 0 walleye populations (Fox 1991)					
15:00, 18:00, and 21:00	0.320	0.020	0.660	0.005±0.013	1.331±1.030
Age 0 yellow perch (Post 1990)					
15:00, 18:00, and 21:00	0.054	0.046	0.900	0.010±0.007	0.658±0.285

Note: For the test pond, $\bar{F}_{24}$ was predicted by incorporating time-specific point estimates of food weights (F_t) measured in walleye collected from the test pond from one, two, and three time periods into the multiperiod model. For the four ponds and Lake St. George, $\bar{F}_{24}$ values were predicted by incorporating F_t values from 15:00, 18:00, and 21:00 into the multiperiod model and then averaging to obtain a mean $\bar{F}_{24}$ for each pond (Fox 1991) or date (Post 1990). Bonferroni joint CIs for the null hypothesis of an intercept (β_0) of 0 and a slope (β_1) of 1 are also provided. Estimates of β_0 and β_1 were derived using the geometric mean regression method.

dom variations (proportion of MSE due to random error > 0.967) and not due to errors in the mean and slope of regressions of predicted versus observed $\bar{F}_{24}$ (Table 3). Bonferroni joint confidence intervals for all but the 15:00 and 18:00 time period combination revealed that regressions of predicted on observed $\bar{F}_{24}$ values were not different from the $y = x$ line (1:1 line) (Table 3). Overall, results indicated that regressions of predicted on observed $\bar{F}_{24}$ values were not significantly different from the 1:1 line (Table 3). Although these procedures tested for the model's ability to accurately predict $\bar{F}_{24}$ for a walleye population in an independent pond, the model ponds and independent test pond were not that different due to similarities in fish densities, water temperature, fish body size, geographic area, and other environmental conditions. I therefore compared multiperiod model predictions of $\bar{F}_{24}$ values with observed $\bar{F}_{24}$ values reported by Fox (1991) for age 0 walleye in four distinct ponds separated from the model ponds by geographical area and time.

This study showed that the low-effort model provided reasonable estimates of $\bar{F}_{24}$ for age 0 walleye in distinct pond systems (Fox 1991). Partitioning of MSE revealed that deviations of predicted $\bar{F}_{24}$ values from observed values were mostly attributed to random errors (proportion of MSE due to random errors = 0.66), although 32% of the errors were attributed to the mean component (Table 3). This indicates that model predictions of $\bar{F}_{24}$ were not as accurate for these distinct ponds in Fox's (1991) study as they were for the independent test pond in this study. However, the Bonferroni joint confidence interval test revealed that regressions of predicted on observed $\bar{F}_{24}$ values were not significantly different from the 1:1 line (Table 3). The deviations between predicted $\bar{F}_{24}$ values and observed $\bar{F}_{24}$ values may be due to differences in sizes and diet of walleye and the methods used to estimate food mass in fish guts between this study and Fox's (1991). Walleye in this study fed primarily on zooplankton, while those in Fox's (1991) study ate mainly chironomids. The maximum mean wet mass of walleye in this study was 350.6 mg, while walleye in two of four ponds in Fox's (1991) study had mean wet masses of 548.0 and 711.0 mg. Fox (1991) determined food mass in walleye guts using length–weight regressions for various prey types, while food mass in walleye guts were estimated as differences in mass of whole guts with food and empty guts (Madon and Culver 1993). In spite of these differences in fish diet and sizes and methods used to estimate food mass in fish guts, the model performed reasonably well.

If the regression model approach relies on fundamental relationships between F_t values and $\bar{F}_{24}$ values, then a model developed for one species should be applicable to another species with similar feeding behavior (i.e., diurnal feeders). Therefore, I used the low-effort model to estimate $\bar{F}_{24}$ values in Lake St. George age 0 yellow perch on seven separate dates (Post 1990). Partitioning of MSE revealed that deviations of predicted $\bar{F}_{24}$ values from observed values were mostly attributed to random errors (proportion of MSE due to random errors = 0.90), and only 10% of the errors were attributed to the mean and slope component (Table 3). This indicates that model predictions of $\bar{F}_{24}$ were generally accurate for age 0 yellow perch. However, the Bonferroni joint confidence interval test revealed that regressions of predicted on observed $\bar{F}_{24}$ values had a slightly higher intercept

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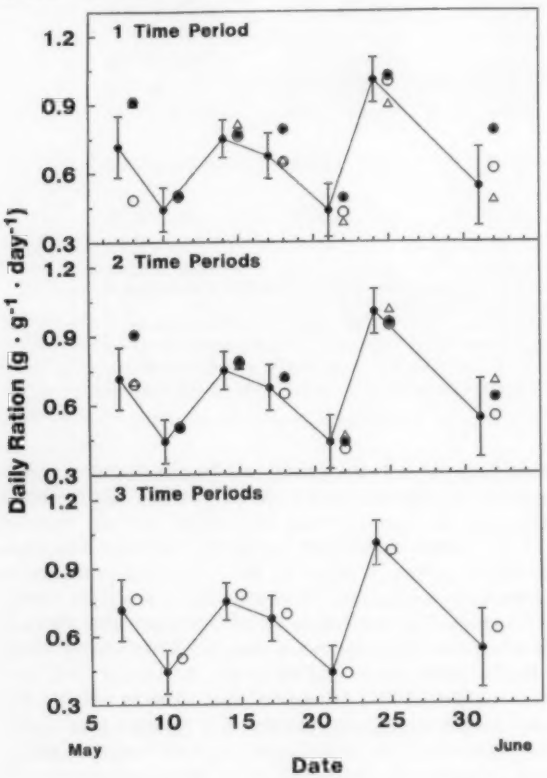
Fig. 2. Comparisons of age 0 walleye daily ration (± 95% CI) in the test pond derived via the E-P method (●, solid line) with daily ration estimates derived from multiperiod model predictions of average food weights ($\bar{F}_{24}$) when point estimates of food weight (F_t) from one to three time periods were included in the model. Symbols in each case represent the following time periods: one time period: 15:00 (○), 18:00 (△), 21:00 (✱); two time periods: 15:00 and 18:00 (○), 15:00 and 21:00 (△), 18:00 and 21:00 (✱); three time periods: 15:00, 18:00, and 21:00 (○). Symbols indicating model estimators are offset to the right of observed values for clarity.

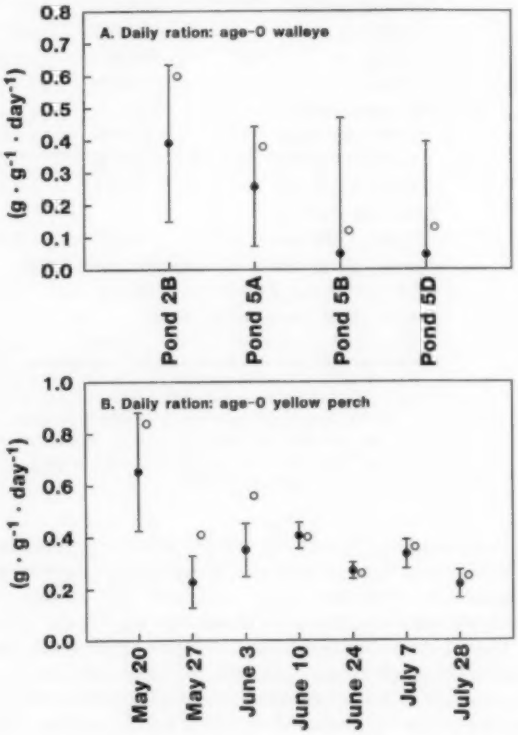


and lower slope than the 1:1 line (Table 3) because the model tended to overpredict $\bar{F}_{24}$ values on five of the seven dates.

Walleye daily ration

I compared values of daily ration of walleye and yellow perch estimated from multiperiod regression model predictions of $\bar{F}_{24}$ (henceforth called predicted daily ration) with daily ration values derived via the standard Elliott and Persson (1978) procedure (henceforth called E-P estimates). In the independent test pond, predicted daily ration estimates were all within the 95% CIs of E-P estimates across all dates when F_t data from three time periods were used in the model (Fig. 2). Hayward et al. (1991) have similarly shown good agreement of daily ration estimates derived via the multiperiod regression model approach with E-P estimates for yellow perch. Predicted daily ration estimates fell outside the 95% CIs of E-P estimates for May 7 and May 31, and

Fig. 3. Comparisons of daily ration predicted by the multiperiod model (○) with daily ration (± 95% CI) observed (●) in (A) age 0 walleye in four ponds that were geographically and temporally distinct from those in this study (Fox 1991) and (B) age 0 yellow perch in Lake St. George, Canada (Post 1990). Pond nomenclature and dates follow those in Fox (1991) and Post (1990), respectively. Symbols indicating model estimators are offset to the right of observed values for clarity.



for May 7, when F_t data from only one time period and two time periods, respectively, were used in the model, but not when three time periods were used (Fig. 2). This indicates that data from a minimum of three time periods must be included to obtain accurate estimates of daily ration. I note that May 7 represents a date that lies outside the period (May 10 – June 4) when data were collected to develop the multiperiod model (Table 1). Consequently, walleye in the test pond on May 7 were of approximately half the mean mass of those in the model ponds on May 10 (Table 1). Daily ration estimates of the model for walleye in all four ponds in Fox's (1991) study were within the 95% CIs of observed values; mean wet masses of walleye in ponds 5B and 5D were more than 1.5–2.0 times the mean wet mass of the largest fish in the model ponds. The model also provided reliable estimates of daily ration for age 0 yellow perch 1.5–4.5 times the size of the largest walleye in the model ponds (Post 1990; Fig. 3, July 7 and 28, this study). This suggests that the regression model can accurately predict daily ration for fish sizes outside that over which the model was constructed. This is the first study to demonstrate the model's ability to tolerate extrapolations to out-of-domain size-classes, although within the same life stages (larvae and ju-

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veniles). Hayward et al. (1991) reported their model's ability to estimate outside of the regression model temporal domain but did not evaluate effects of fish size. However, use of the model to predict daily ration in walleye larger than those in this study should proceed with caution. The fundamental mathematical relationships on which the model relies will differ in larger fish, and a separate model would thus be required for larger walleye. The same applies to bioenergetics models where separate models are needed for age 0 and adult walleye due to vast size-specific differences in energetic parameters utilized by these models (Madon and Culver 1993).

This study demonstrates that the low-effort approach yields a model that can reliably predict $\bar{F}_{24}$ and daily ration for geographically and temporally distinct populations of age 0 walleye. The ability of the age 0 walleye model to provide daily ration estimates within 95% CIs of five out of seven observed daily ration values for age 0 yellow perch in Lake St. George (Fig. 3) lends further evidence for the general applicability of this approach in fish consumption studies. However, the applicability of the model to lake and reservoir walleye populations warrants further direct testing. Existing F_t and $\bar{F}_{24}$ data collected as part of 24-h feeding studies of lake or reservoir walleye could be used in the low-effort walleye model to test its applicability to walleye populations in these systems. Based on the above results for age 0 walleye in distinct ponds, and age 0 lake yellow perch, it seems likely that the walleye model developed in this study could be reliably applied to lake and reservoir walleye.

The sizes of walleye in this study represent a critical and dynamic stage in early fish life history. Consumptive demands by fish on food resources during these life stages vary tremendously on a temporal and spatial basis due to rapid growth (Houde 1987; Madon 1993; Madon and Culver 1993), and any in-depth study on early life dynamics needs to account for this variability and complexity. The low-effort regression approach shows tremendous promise as a way to reduce effort and increase the spatial scope in field studies of fish food consumption.

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Spatial and temporal factors affecting survival in coho salmon (*Oncorhynchus kisutch*) in the Pacific Northwest

Claribel Coronado and Ray Hilborn

Abstract: Survival rates for coho salmon (*Oncorhynchus kisutch*) were estimated for all coded wire tag release groups in the Pacific Northwest between 1971 and 1990. The spatial and temporal patterns show considerable geographic variation, with most regions south of northern British Columbia showing declining survival since 1983, while northern areas have shown increasing survival during that period. The number of years of operation explained very little of the variation in survival, and many hatcheries showed major increases in survival after several years of operation. Survival of marked wild fish generally showed the same trend as hatchery fish. We conclude that the dominant factor affecting coho salmon survival since the 1970s is ocean conditions and that there are major geographic differences in the pattern of ocean conditions. The decline in survival seen in British Columbia and south over the last decade suggests that a major reduction in exploitation rates is necessary to maintain the populations.

Résumé : Le taux de survie a été estimé pour tous les groupes de saumons cohos (*Oncorhynchus kisutch*) marqués par fil codé et libérés dans le nord-ouest du Pacifique entre 1971 et 1990. Les allures spatiales et temporelles présentent une variation géographique considérable. On a noté, depuis 1983, un déclin du taux de survie dans la plupart des régions situées au sud de la partie nord de la Colombie-Britannique, mais une augmentation de ce taux dans les régions situées au nord. Le nombre d'années d'exploitation explique très peu de la variation du taux de survie et bon nombre de pisciculture présentaient d'importantes augmentations de la survie après plusieurs années de fonctionnement. La survie des poissons sauvages marqués présentait généralement une allure semblable à celle des poissons d'élevage. Nous concluons que le facteur qui a le plus influé sur la survie du saumon coho depuis les années 1970 est représenté par les conditions océaniques et que ces conditions présentent d'importantes différences en fonction du lieu géographique. Le déclin du taux de survie noté en Colombie-Britannique et dans les régions situées au sud au cours de la dernière décennie porte à croire que le maintien des populations exigera une importante réduction des taux d'exploitation.

[Traduit par la Rédaction]

Introduction

Coho salmon (*Oncorhynchus kisutch*) have been produced in hatcheries for over 100 years (Cobb 1931; WDF 1991). During the early years, rearing success was limited, but by 1965, there were 23 facilities in the United States and Canada releasing over 21 million fish annually. Concerns about population declines promoted an increase in hatchery production of salmonids, and by 1989, 420 hatcheries existed in the same region, releasing 147.8 million coho fry (Fig. 1). Hatchery production of coho has presently reached high proportions, and an estimated 40–50% of net-caught salmon in Puget Sound are hatchery reared (Laufle et al. 1986).

During the long history of hatchery production of salmonids, efforts have been made to improve rearing techniques, leading to an increase in the number of fish released. An initial focus on increasing the number of fish released has been questioned in the last two decades when it has become clear that an increase in the number of juveniles released does not necessarily mean an increase in number of adults recovered. Many factors have been proposed to explain variation in coho including changes in ocean conditions, predation at sea, density-dependent mortality, poor quality of hatchery smolts, and diseases (Pearcy 1988, 1992; Walters 1988). Correlations with ocean conditions have been intensively studied for the Oregon Production Index (OPI) area (Fig. 2). Upwelling and ocean temperatures have been correlated with smolt-to-adult survival of hatchery fish, while the correlations are weaker or nonsignificant for wild coho from the same area (Nickelson 1986).

In spite of some significant correlations, evidence supporting the changes in ocean conditions is still inconclusive and the causes of variation in smolt-to-adult survival are still speculative (Pearcy 1992). Upwelling may influence survival by affecting distribution at sea, food abundance, and predator distribution. In the present study, we compare coho survival over a broad geographic range to study geographical and temporal patterns in survival in the Pacific Northwest. Additionally, we explore the relationship between survival and the time that hatcheries have been in operation and test the hypothesis that declines in survival are caused by an increase in production from hatcheries located in less favorable locations.

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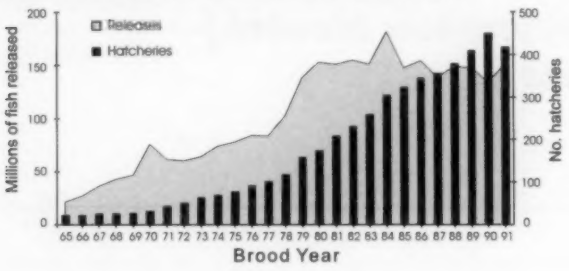
Fig. 1. Number of facilities producing coho in the Pacific Northwest and millions of fish released by brood year.


Releases
Hatcheries
Millions of fish released
No. hatcheries
Brood Year
65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91

Methods

Survival calculation was based on coded wire tag (CWT) recoveries (Jefferts et al. 1963) obtained from the CWT database of the Pacific States Marine Fisheries Commission (Gladstone, Oreg.). To avoid potential bias, only groups classified as nonexperimental were included in the analysis. The database contains information on observed number of tags recovered in a sample fishery and the estimated number of tagged fish, or expanded recoveries, that the recovery represents. Because most fisheries are sampled at different rates, our analyses were based on expanded recoveries. Throughout this study, hatchery refers to rearing facilities and age is calculated as recovery year minus brood year. CWT coho have been recovered at ages ranging from 1 to 7 years, but along this study's entire geographical distribution, 91% of fish return at age 3. Although most recoveries are made at age 3, the age distribution at recovery varies among the different regions. Because of this variation, standardization of the number of fish recovered was necessary to compare recoveries among regions. To standardize the recoveries, age 3 was chosen as the standardization age and a combination of forward and backward virtual population analysis (VPA) (Hilborn and Walters 1992, chap. 10) was used:

$$N_3^* = C_1 s_1 s_2 + C_2 s_2 + C_3 + \frac{C_4}{s_4} + \frac{C_5}{s_4 s_5} + \frac{C_6}{s_4 s_5 s_6} + \frac{C_7}{s_4 s_5 s_6 s_7}$$

where N_3^* is the number of fish that would be alive if they were not caught or escaped at an age different from age 3, C_t is the number of fish recovered (caught plus escaped) at age t, and s_t is the survival rate from age $t-1$ to age t (which can also be expressed as 1 minus mortality at age t). The mortality rate 0.5 (Argue et al. 1983) was used as a scaling factor and has no impact on the patterns presented and analyzed. Survival for each tag code was calculated as

$$\text{Survival} = \frac{N_3^*}{\text{No. released}}$$

Average survival was calculated as the arithmetic mean of survival for individual tag codes, grouped by hatchery location and (or) by brood year. The standard deviation of the mean was calculated as the ratio of standard deviation of the survival of individual tag groups and the number of tag groups. This statistic was included in the charts as error bars around the mean as a measure of variability of the average survival.

To compare the influence of geographic location of the hatcheries and rearing facilities, each state/province was divided into sectors and each sector into regions. The division into sectors corresponds approximately to the partitioning of management areas, while regions correspond approximately to production areas (Johnson and Thompson 1989). Geographical division by state/province, sector, and region is listed in Table 1. As an example, Washington State was divided into three sectors, Puget Sound, Washington coast, and Columbia River. The Columbia River sector was divided into two regions, lower Columbia River and upper Columbia River.

Fig. 2. OPI area and its components (after ODFW 1982).

To test hypotheses regarding brood year, hatchery location, time of release, hatchery age, and weight at release, a generalized linear model (GLM) was fitted. Hatchery age represents the number of years that a hatchery has been operating. The GLM was log-linear with a scaled Poisson error distribution. Number of expanded recoveries was used as a predicted variable. Details of the model can be found in Green and Macdonald (1987), Cormack and Skalski (1992), and Pascual (1993). The independent variables brood year, hatchery location, and release month were categorical, while hatchery age and weight were used as covariables. The logarithm of the number of fish released was used as a weight, offset in GLM terminology, to account for differences in release numbers for the different tag codes. Changes in deviance were used as goodness-of-fit criteria (Green and Macdonald 1987). The deviance has a distribution that asymptotically approaches a chi-square, so it can be used as a guide for selection among alternative models. Model fitting emphasized the relative importance of the different sources rather than the search for the best model.

For the construction of the model, it was necessary to exclude the regions with the most incomplete history of tagged releases. The selection was made to include regions with at least one group of tagged releases in a minimum of 9 years during the period 1973–1990. Brood year 1973 was chosen as the lower bound of the range because it was then that the number of hatcheries releasing CWT coho started to increase. Brood year 1990 was chosen as the upper limit of the range because it was the last brood year for which complete recovery data were available. A total of 34 re-

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Table 1. Geographical divisions by state/province, sector, and region.

State/province	Sector	Region	Description
AK			Alaska
	AK.CC		Central Alaska
		COOK	Cook Inlet
		KODK	Kodiak Island
		PWS	Prince Williams Sound
	AK.SE		Southeast Alaska
		STEP	Stephens Passage
		C.OUT	Central Outside
		S.OUT	Southern Outside
		S.IN	Southern Inside
	ARTC		Arctic
		MYUK	Mid-Yukon
	AK.SEm		Southeast Alaska net pens
		STEPm	Stephens Passage net pens
		SOUTm	Southern Outside net pens
	AK.CCm		Alaska central coast net pens
		COOKm	Cook Inlet net pens
BC			British Columbia
	BC.NO		British Columbia north coast
		CCST	Central coast
		NASS	Nass River
		NCST	North coast
		QCI	Queen Charlotte Islands
		RIVR	River R
		SKNA	Skeena River
		TRAN	Transboundary
		YUKN	Upper Yukon
	BC.SO		South coast of British Columbia
		GSMN	Strait of Georgia mainland north
		GSMS	Strait of Georgia mainland south
		GSVI	Strait of Georgia Vancouver Island
		JNST	Johnston Strait
		LWFR	Lower Fraser River
		NWVI	Northwest Vancouver Island
		SWVI	Southwest Vancouver Island
		TOMF	Thompson River fork
		TOMM	Thompson River mainland
		UPFR	Upper Fraser
WA			Washington
	STWD		Statewide
		STWD	Statewide
	PGSD		Puget Sound
		NOOK	Nooksak–Samish
		SKGT	Skagit
		STILL	Stillaguamish–Snohomish
		HOOD	Hood Canal
		SPGT	South Puget Sound
		STJF	Juan de Fuca Strait
		W.CST	Coastal Washington
		NORC	Washington north coast
		SO.C	Washington south coast
		WILL	Willapa Bay
		W.CR	Columbia River, Washington
		LWC.W	Lower Columbia River, Washington
		UPC.W	Upper Columbia River, Washington
		SNK.W	Snake River, Washington
		PGSDm	Puget Sound net pens
		NOOKm	Nooksak–Samish net pens
		SKGTm	Skagit net pens
		STILLm	Stillaguamish–Snohomish net pens
		HOODm	Hood Canal net pens
		SPGTm	South Puget Sound net pens
		STJFm	Juan de Fuca Strait net pens
		W.CSTm	Coastal Washington net pens
		SO.Cm	Washington south coast net pens
		WILLm	Willapa Bay net pens
ID			Idaho
		SNKE	Snake River
		SALM	Salmon River
		CLWT	Clearwater River
OR			Oregon
		O.CST	Oregon coast
		O.NCS	Oregon north coast
		O.SCS	Oregon south coast
		O.CR	Columbia River, Oregon
		LWC.O	Lower Columbia River
		WILM	Willamette River
		UPC.O	Upper Columbia, Oregon
CA			California
		C.NCS	California north coast
		SMITH	Smith River
		C.CCS	California central coast
		GARC	Garcia River
		EEL	Eel River
		LTRV	Little River
		MAD	Mad River
		RUS	Russian River
		SCSJ	Sacramento, San Joaquin
		SCRM	Sacramento
		SJOAQ	San Joaquin
		C.SCS	California south coast
		SOUTH	South reach
		KLAM	Klamath
		KLAM	Klamath River
		TRNT	Trinity River

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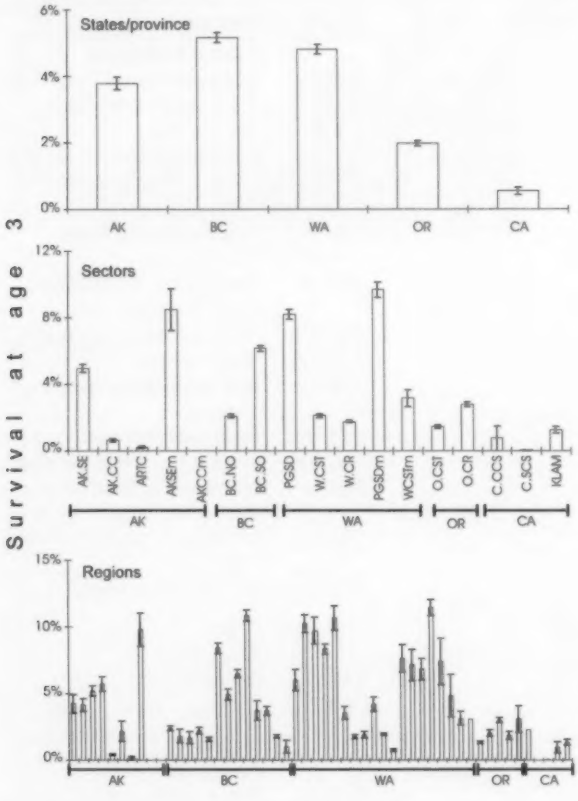
Fig. 3. Average survival of hatchery coho. Error bars indicate standard deviation of the mean.



gions, distributed among 12 sectors and four states and one province, complied with the selection criteria.

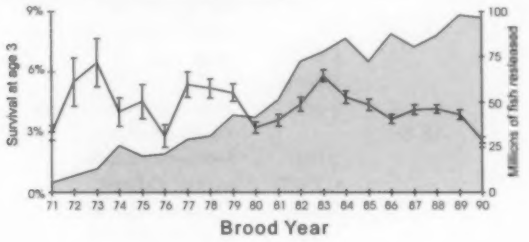
A cluster analysis compared survival trends in different hatcheries and tested the hypothesis that hatcheries from the same regions show similar survival trends. Average survival by brood year was tabulated, and because of the requirements for the cluster analysis, only hatcheries without missing data were included. The options ranged from a large number of brood years (longer trends, better data for clusters) but few hatcheries to a small number of brood years but more hatcheries. An intermediate option was selected to include as many hatcheries with as many brood years as possible. The cluster analysis was performed using the software package SPSS. The cosine coefficient measured similarity between hatcheries. Clusters were formed using the average distance between clusters (UPGMA) as the clustering algorithm.

Results

The average survival by state/province aggregated over time (Fig. 3) ranged between 6.21% in British Columbia and 0.92% in California with a mean survival of 4.27%. British Columbia, Washington, and Alaska exhibit the highest survival rates, all of them with averages over 3.7%.

The differences among areas are larger at the sector and region level. At the sector level, three areas, southeast Alaska (hatcheries and net pens), Puget Sound (hatcheries and net pens), and the south coast of British Columbia, all have mean survivals higher than 5%. At the region level, the differences are very strong. There are 21 regions with mean survivals higher than 4%, and eight of those show survivals above 8%. There are also many regions with mean survivals of 2% or below. Regions with poor survival are found across the entire geographic range but are more common in California, where only the Mad River facilities have a mean survival above 2%. Poor survivals are also common in central Alaska and on the north coast of British Columbia.

Fig. 4. Temporal trends in coho aggregated over the entire range. The line represents survival and error bars are standard deviation of the mean. The shaded area and right-hand axis represent millions of fish released by brood year.



Trends

Temporal trends of survival aggregated over the entire study area (Fig. 4) show alternating increases and declines from 1971 to 1983. After 1983, the survivals declined, reaching an average below 3% in 1990, which is the last year included in the analysis. In sharp contrast, releases steadily increased since the beginning of the period, reaching the maximum in 1989 and 1990.

Production of hatchery coho in the different areas (Fig. 5) averaged 20 000 000 to 30 000 000 fish per year, with the exception of California which produced between 300 000 and 600 000 fish per year. When survival trends are examined at the state level, we see a basic pattern of declining survivals since the mid-1980s. Alaska is the only exception, showing two peaks in survival. The first one occurred in the 1980–1982 broods, with declines in the 1983–1985 broods and increasing survivals for the 1986–1989 broods. In British Columbia, the survival trend for coho is characterized by high survivals (around 12%) during the early 1970s when only a few groups were tagged and released. After brood year 1975 came a steady decline and subsequent stabilization at about 5%. Trends for Washington show periods of moderately high and moderately low survivals, with a tendency of stabilization around 5%. Survivals declined after brood year 1987. Oregon and California show low survivals when compared with the other states and province. In Oregon the maximum observed survival has been a little over 4%, while in California, it has been close to 2%. Survival trends in Oregon and California are not steady, alternating between years with high and low survivals during most of the period of study.

The two Alaskan sectors in which coho are produced and tagged are southeast and central Alaska (Fig. 6). In southeast Alaska, less than 3% of the coho produced are reared in net pens. When comparing trends from hatcheries and net pens, it is noticeable that even though the net pens showed considerably higher survival, the overall trends are very similar

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Fig. 5. Survival trends for Coho. The line represents survival and error bars are standard deviation of the mean. The shaded area is number of fish released by brood year × 100 000.

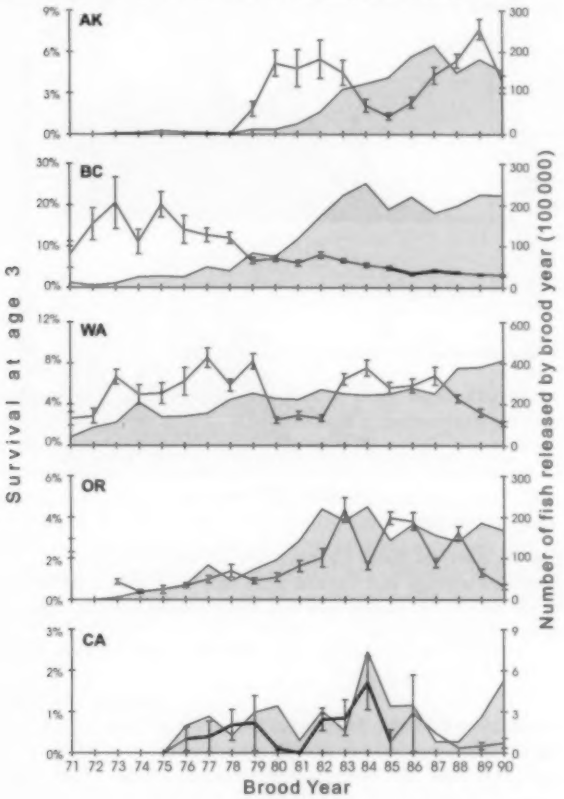
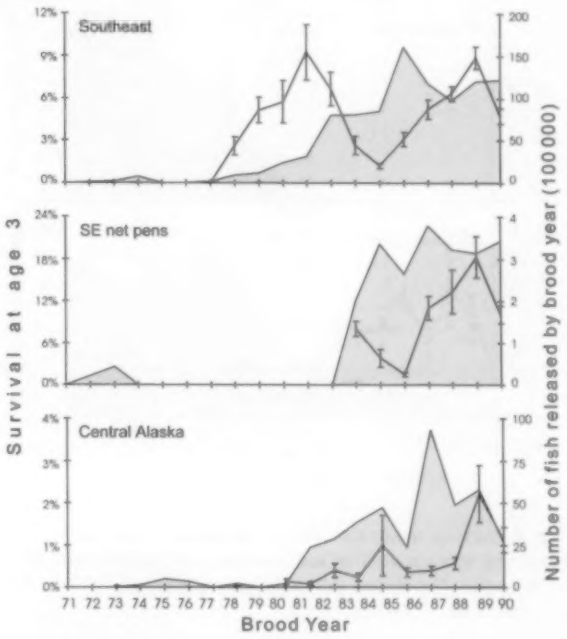

AK
BC
WA
OR
CA
Survival at age 3
Number of fish released by brood year (100 000)
71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90
Brood Year

Fig. 6. Survival trends for coho in Alaska. The line represents survival and error bars sre standard deviation of the mean. The shaded area represents number of fish released by brood year × 100 000.


Southeast
SE net pens
Central Alaska
Survival at age 3
Number of fish released by brood year (100 000)
71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90
Brood Year

with a low in the mid-1980s followed by a rebound in the second half of the 1980s and a drastic drop in 1990. In central Alaska, survivals are considerably lower, but the overall survival trend shows a pattern very similar to the one observed in southeast Alaska.

In British Columbia (Fig. 7), coho salmon were produced only on the south coast during the 1970s. During that early period the annual production of coho was below 5 million fish per year, but the observed survivals were above 10%. In the 1980s, coho production on the south coast was increased, and by 1982 the annual production was above 15 million fish. Survivals, on the other hand, did not increase with production, showing a steady decline since 1975 and a tendency to stabilize at around 5%. During the early 1980s, along with increased production on the south coast, hatcheries on the north coast of British Columbia also began to rear and tag coho. The production level on the north coast was smaller, reaching a peak in 1989 when 35 million fish were reared. Survivals were below 4%.

In Washington (Fig. 8), the three sectors into which the state was divided have produced coho since 1965. Production increased in all three sectors during the 1970s and 1980s, but the increase is more noticeable in the Columbia River basin where by 1989, coho production was over 31 million fish per year. Puget Sound exhibits the highest survivals in the state with rates that often are double the rates from the other two sectors. Coho are produced in hatcheries and net pens, the latter showing a slightly higher survival rate. Since the mid-1980s, survivals in Puget Sound have been declining. The Washington coast shows alternating years of high and low survival with an average close to 2%. Net pens on the Washington coast released coho on a small scale during the early 1970s and then on a larger scale since 1987. These net-pen-reared tag groups show a slightly better survival. Coho production in the Columbia River basin in Washington shows survivals around 2% with a period of above-average survivals in the mid-1980s and a subsequent decline since 1988.

In Oregon (Fig. 9), coho are produced in the coastal area and in the Columbia River basin. In the Oregon coast sector, survivals showed an increase during the early 1980s, reaching a peak of 4% for the 1983 brood year. Since 1985, survivals have been steadily declining. On the Oregon side of the Columbia River basin, alternating years of high and low survivals are observed, a trend that is very similar to the one observed on the Washington side of the basin.

In California (Fig. 10), coho are produced on the south coast and in the Klamath River basin. California production of coho has been very small compared with production in the other states and province. During 1990, the production level sharply increased in the Klamath River basin. Survival rates from the south coast of California are minimal with an average close to 0.05%. In the Klamath area, survivals are higher, but still below average when compared with the other states and province.

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Fig. 7. Survival trends for coho in British Columbia. The line represents average survival and error bars are standard deviation of the mean. The shaded area represents number of fish released by brood year × 100 000.

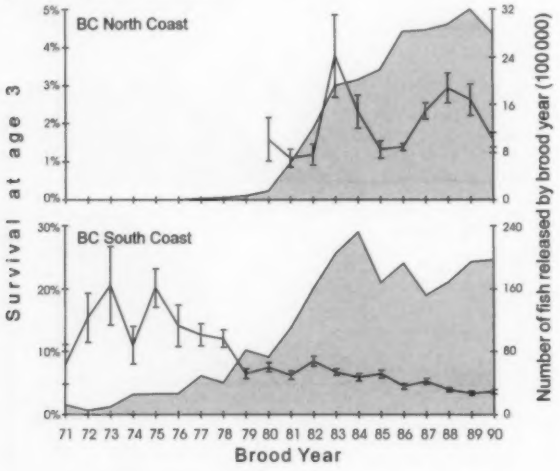


Fig. 8. Survival trends for coho in Washington. The line represents average survival and error bars are standard deviation of the mean. The shaded area represents number of fish released by brood year × 100 000.

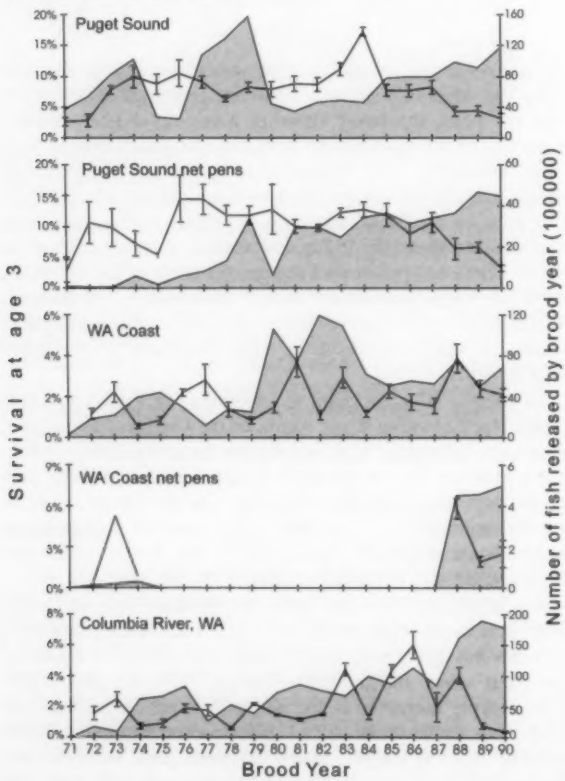


Fig. 9. Survival trends for coho in Oregon. The line represents average survival and error bars are standard deviation of the mean. The shaded area represents number of fish released by brood year × 100 000.

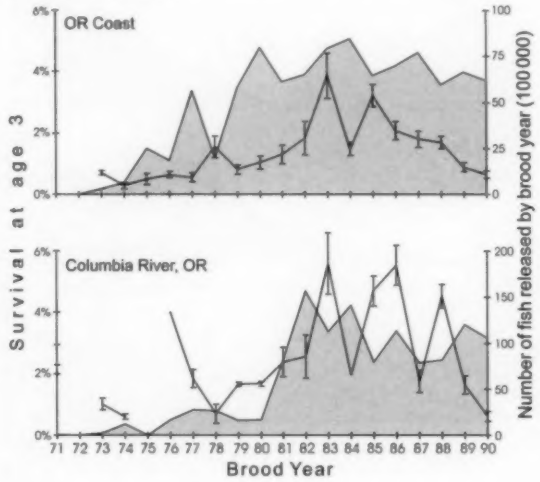
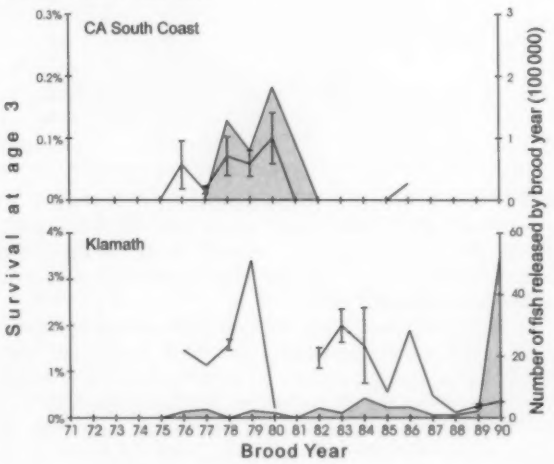


Fig. 10. Survival trends for coho in California. The line represents average survival and error bars are standard deviation of the mean. The shaded area represents number of fish released by brood year × 100 000.



GLM

A GLM was fitted by comparing nine alternative models. The data set tested consisted of over 3000 records, requiring large computing capabilities. The data set is overdispersed and shows high variability. In the first step, six alternative models, each of them containing one main factor, were tested (Table 2). From the six main factors studied, the three factors corresponding to geographical location (state/province, sector, and region) were the ones that explained the highest amount of variability. Since these three factors are nested, only one of them could be included at any time. The factor "region" was chosen because it explained a larger

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Table 2. Summary of GLM analysis to study the relative importance of different factors affecting survival.

Model	Action	Dev.	df	Scaling factor	Scaled Δdev.	Δdf
Brood (b)		3 397 328	3655	929.50	175.00	16
State		3 285 669	3667	896.00	306.20	4
Sector		2 372 143	3660	648.10	1832.90	11
Region (rg)	*	1 912 891	3638	525.80	3132.60	33
Release month (rl)		3 308 226	3660	903.90	278.60	11
Weight (w)		3 516 643	3670	958.20	45.30	1
rg + b	*	1 774 990	3622	490.10	281.40	16
rg + rl		1 768 262	3627	487.50	296.68	11
rg + w		1 888 758	3637	519.30	46.47	1
rg + b + rl		1 627 785	3611	450.00	326.50	11
rg + b + w		1 746 963	3621	482.50	58.10	1
rg + b + rg·b	wk full					

amount of variability. In the second step, three alternative models were compared, each containing region plus another main factor. At this second level, both brood year and release month explained approximately the same amount of variability, while average weight at release had very little impact on the deviance. At the third level, alternative models with three main factors or with two main factors and an interaction term were tested. It was not possible to test the interaction because of lack of computer capability, but the model where the factors "region ," "brood year ," and "release month" were included was the most significant.

Cluster analysis

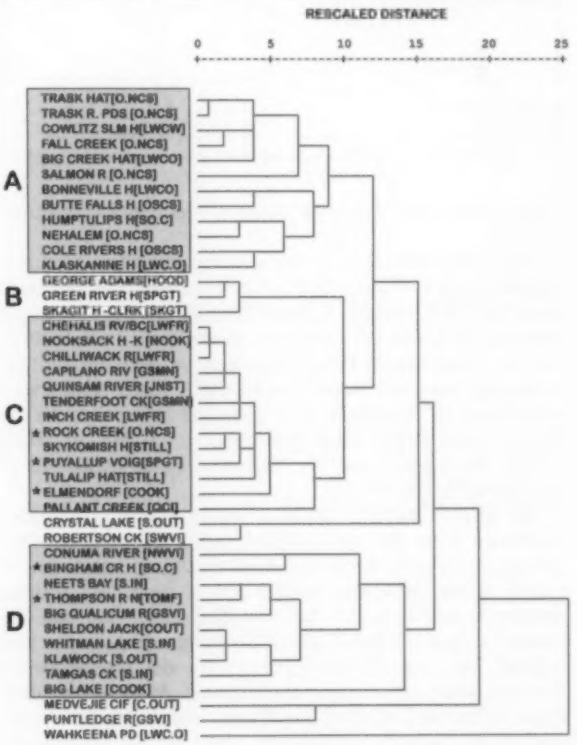
Using cluster analysis, 43 hatcheries were grouped in six clusters (Fig. 11). Hatcheries within clusters tended to be located in the same geographical area (Fig. 12), although some variability can be observed. Cluster A comprises 12 hatcheries, four of them located in the lower Columbia River and seven in coastal Oregon. Cluster B is formed by three hatcheries located in Puget Sound. Cluster C groups 13 hatcheries, six from the protected waters on the south coast of British Columbia and four from Puget Sound. Cluster D is formed by 10 hatcheries, five located in southeast Alaska and three on the south coast of British Columbia.

Discussion

The results show an increase in hatchery production coupled with a slight downward trend in survival when coho production is pooled over the entire geographic range. Grouping tag codes by location of the hatchery, an increase in survival during the mid- and late 1980s is evident in northern regions, central Alaska, southeast Alaska, and northern British Columbia. Conversely, declines in survivals are observed in all the other areas with the exception of coastal Washington where survivals appear to remain stable at around 2%.

The difference in survival trends between Alaska and the southern regions can be explained based on the concept of three major production domains identified in the northeast Pacific Ocean (Ware and McFarlane 1989). Alaskan stocks are located in the coastal downwelling domain, while southern stocks are in the coastal upwelling domain. Northern

Fig. 11. Clustering of coho hatcheries using release trends from brood years 1982–1990. Rescaled distance indicates the proportional distance among groups. A star indicates out of the group. See Fig. 12 and Table 1 for locations and abbreviations.



British Columbia stocks, on the other hand, are located in a transition zone, which could explain the differences between the north and south coasts of British Columbia.

Previous studies have reported population declines in coho. Cross et al. (1991) studied survivals from British Columbia hatcheries from 1975 to 1985 and found a decline in survival since 1975 but a significant recovery in 1985. A continued decline is evident with the inclusion of 5 more

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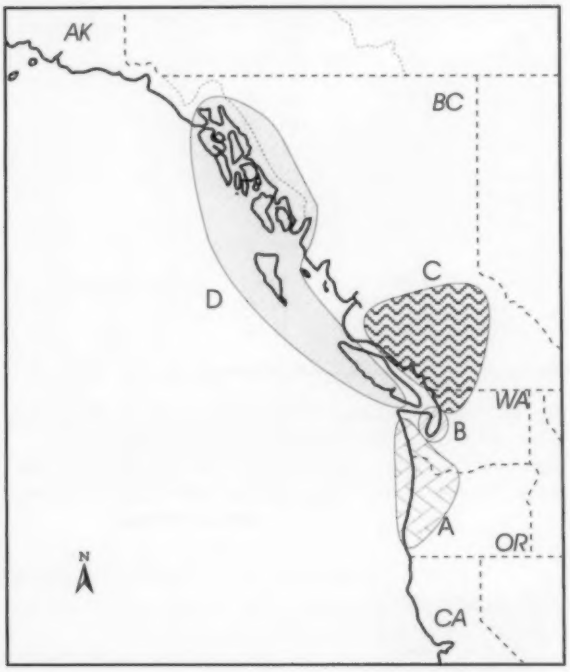
Fig. 12. General location of the groups resulting from the cluster analysis.

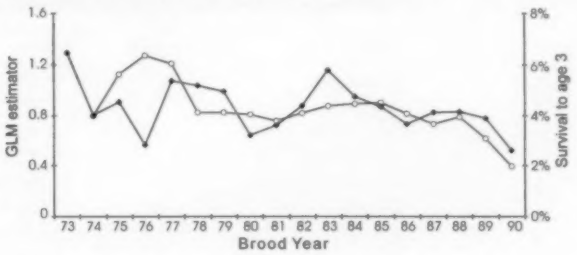


years of data (Fig. 7) and no signal of recovery has been observed.

Within the OPI (Fig. 2) the general perception (summarized by Pearcy 1992) is that an increase in coho production since the 1960s resulted in large fluctuations in adult returns between 1970 and 1975 and low returns from 1976 to 1986 with the exception of 1985 where high numbers of adult returns were observed. Recoveries of CWT, which started with brood year 1973, indicate that a decline in survival on the Oregon coast started after brood year 1983 (Fig. 9), while coastal Washington and the Columbia River continued to fluctuate (Figs. 8 and 9).

The cluster analysis detected higher similarities among hatcheries from the same region, indicating a geographic pattern in survival. From the dendrogram, all hatcheries in cluster A are influenced by the coastal upwelling regime, clusters B and C are in the transition zone, and half the hatcheries from cluster D are in the downwelling production regime. The most successful areas for the production of hatchery coho are evident in southeast Alaska, the south coast of British Columbia, and Puget Sound. All these areas tend to be located towards the center of the distribution range of coho whereas hatcheries that are located at either extreme of the distribution range tend to have poor survivals. Southeast Alaska, southern British Columbia, and Puget Sound are also areas where smolts first enter protected areas rather than directly entering the open ocean; these protected areas probably contribute to a higher survival rate (Nickelson and Lichatowich 1984). The higher survival for coho produced towards the central area of the geographical range was also reported by Pearcy (1992).

Fig. 13. Comparison of average survival and brood year effect from the GLM. ◆, mean survival; ○, estimate.



On a temporal scale, no period has better survivals overall, but some periods have good survivals for specific regions when the spatial scale is taken into consideration. For example, 1985–1989 were good brood years for Alaska, while the early 1970s were very good brood years for southern British Columbia. In terms of climatic changes, a regime shift has been identified in the mid-1970s (Graham 1994); some variability in salmon production has been explained based on this change in ocean conditions (Hare and Francis 1995). Unfortunately, CWT mark–recapture programs started only after 1971, which does not allow us to test for these long-scale variations.

Declines in survival could also be explained as a consequence of increased production from new hatcheries located in less favorable areas. Space in the preferred areas was limited, so new hatcheries were built in less favorable locations and fish reared there have lower survival. The opening of more hatcheries located in less favorable locations was thought to have increased the proportion of "low-survival fish" and to have driven survival trends downward. To explore the validity of this hypothesis, a GLM was fitted with region and brood year effects. The estimate of the brood year effect from that model was plotted and compared with the overall survival trends (Fig. 13). The very similar trends of both lines indicate that declines in survival trends are independent from the hatchery location, since that effect is already accounted for in the GLM.

Another common hypothesis relates declines in survival to the number of years that a hatchery has been operating, i.e., the older the hatchery the lower the survival (Walters 1988). Suggested causes have been disease buildup in the facilities and genetic deterioration of the stock. From the GLM analysis, it is evident that survival does not decline as a function of the number of years that the hatchery has been in operation. There are variables, such as hatchery location and brood year, that are orders of magnitude more significant for explaining the variation in survival (Table 3).

Similarities in survival trends between net pen groups, hatchery groups, and wild fish were noticeable. Net pen groups are reared mostly in marine water environments and are not held in tanks and ponds typical of hatchery-reared fish. Net pen reared fish usually have a higher survival rate, but survival trends are very similar to those of their counterpart hatchery fish. In regions where wild coho have been marked with CWT, both wild and hatchery fish show very similar survival trends (Figs. 14 and 15). Survival rates for wild fish are possibly underestimated because of the limited tag recovery programs for spawning grounds. Even with the

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Table 3. Summary of the GLM analysis to test the "hatchery age" effect.

Model	Action	Dev.	df	Scaling factor	Scaled Δdev.	Δdf
Region (rg)	*	1 035 002	1971	525.12	2261	31
Brood (b)		1 974 154	1985	994.54	994.5	17
Release month (rl)		1 983 086	1995	994.03	240.8	7
Hatchery age (hg)		2 220 025	2001	1109.46	2.145	1
Weight (w)		2 221 996	2001	1110.44	0.3683	1
rg + b	*	904 347	1954	462.82	282.3	17
rg + rl		996 646	1964	507.46	75.58	7
rg + hg		1 025 034	1970	520.32	19.16	1
rg + w		1 034 243	1970	525.00	1.446	1
rg + b + rl		866 160	1947	444.87	85.84	7
rg + b + hg		875 094	1953	448.08	65.29	1
rg + b + w		902 230	1953	461.97	32.43	1
rg + b + rg·b	wk full					

Fig. 14. Survival trends of hatchery and wild coho in southeast Alaska and British Columbia. ◆, mean survival; ○, estimate.

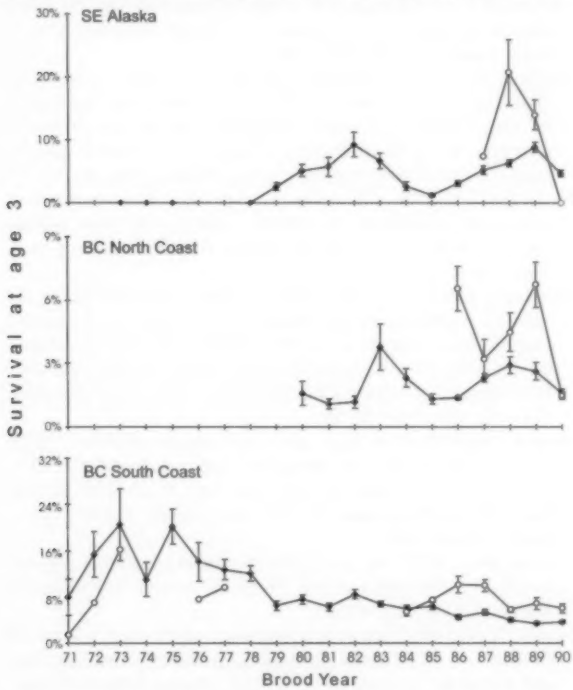
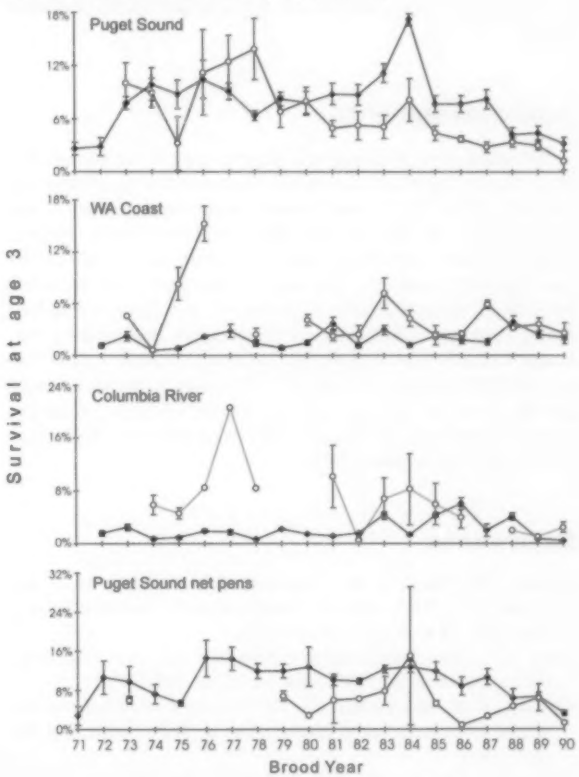


Fig. 15. Survival trends of hatchery and wild coho in Washington. ◆, mean survival; ○, estimate.



underestimation of wild survival rates, wild fish normally exhibit higher survival rates than hatchery fish. Observing the similarities in survival trends within regions between wild, net pen, and hatchery coho, it is likely that ocean conditions have a strong effect on the survival of coho.

In British Columbia (Fig. 7), there is a marked decline in survival when the number of fish released increases whereas this relationship is less clear in Washington, Oregon, and California and apparently nonexistent in Alaska. The negative relationship between survival and number of fish released supports the idea of density dependence, but the evidence is weak, and the effect is confounded by the major climate shift in the late 1970s. The density dependence hypothesis has been investigated by McCarl and Rettig (1983), Peterman and Routledge (1983), McGie (1984), Nickelson (1986), and Emlen et al. (1990), but there is still no consensus on whether or not it is responsible for the decline of coho populations. Making relationships more difficult to understand is the difference in survival of hatchery and wild

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coho. As pointed out by Nickelson (1986) and Emlen et al. (1990) and confirmed in this study, wild coho have better survival rates than hatchery coho. This difference, coupled with the change in stock composition from mainly wild fish to a majority of hatchery fish, makes it difficult to analyze the influence of other factors such as density dependence.

The similarities in survival trends of hatchery, net pen, and wild coho in the different areas strongly support the idea of the importance of ocean conditions on survival to adult. These changes in ocean conditions also make it more difficult to interpret the impact of human action. Pending possible return to more productive oceanographic conditions, rebuilding coho populations in the southern range is dependent on modifications of management in harvest and production. Declines in ocean survival lower the sustainable exploitation level; for example, in British Columbia the survival is now only one quarter of what it was in the early 1970s. This calls for a drastic reduction in or total cessation of exploitation. A reduced harvest needs to be matched by an increasing reliance on natural production and habitat restoration to maintain the existing genetic diversity required for long-term perpetuation of the species in its southern range.

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Hepatic mixed function oxygenase activity and vitellogenin induction in fish following a treatment of the lampricide 3-trifluoromethyl-4-nitrophenol (TFM)

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Abstract: Recent laboratory studies with nontarget fish species have shown that the lampricide 3-trifluoromethyl-4-nitrophenol (TFM) exhibits estrogenic activity through binding to rainbow trout (*Oncorhynchus mykiss*) hepatic estrogen receptors and induction of vitellogenin in hepatocyte cultures. In addition, mixed function oxygenase (MFO) activity associated with exposure to field formulations has been attributed in part to the presence of chloro-nitro-trifluoromethyl-dibenzo-*p*-dioxin impurities. To investigate the environmental effects associated with these findings, the temporal and spatial patterns of MFO activity and vitellogenin induction were monitored in three nontarget fish species following a TFM field treatment. Elevated MFO activity was detected as early as 1 day in caged rainbow trout and activity in trout, wild white sucker (*Catostomus commersoni*), and longnose dace (*Rhinichthys cataractae*) peaked 2 or 3 days after treatment. Highest activities were observed in fish exposed closest to lampricide application points and declined with increasing distance downstream. After 18 days, MFO activity was reduced but remained almost sevenfold reference values at several sites. Plasma vitellogenin was not detected in caged trout sampled 6 and 18 days post-treatment, and dioxin impurities were not detected in sediments after treatment. It was concluded that laboratory testing underestimated the duration of MFO activity under field conditions and that an assessment of formulation exposure during sensitive life stages represents an area for further study.

Résumé : Des études récentes menées en laboratoire sur des espèces de poissons non cibles ont montré que le lampricide 3-trifluorométhyl-4-nitrophénol (TFM) présente une activité oestrogénique en se liant aux récepteurs hépatiques d'oestrogènes de la truite arc-en-ciel (*Oncorhynchus mykiss*) et par l'induction de la vitellogénine dans des cultures d'hépatocytes. De plus, l'activité de l'oxydase à fonction mixte (OFM), associée à l'exposition à des formulations *in situ*, a été attribuée en partie à la présence d'impuretés de chloronitrotrifluorométhyldibenzo-*p*-dioxine. Afin d'étudier les effets environnementaux associés à ces résultats, on a surveillé les profils spatio-temporels de l'activité de l'OFM et de l'induction de la vitellogénine chez trois espèces de poissons non cibles après un traitement *in situ* au TFM. On a décelé une forte activité de l'OFM après un jour seulement chez la truite arc-en-ciel en cage, et l'activité chez la truite et chez des spécimens sauvages de meunier noir (*Catostomus commersoni*) et de naseux des rapides (*Rhinichthys cataractae*) atteignait un maximum 2 à 3 jours après le traitement. Les plus fortes activités étaient observées chez les poissons exposés les plus près des points d'application du lampricide, et elles diminuaient vers l'aval proportionnellement à la distance. Après 18 jours, l'activité de l'OFM était réduite, mais à plusieurs sites, elle restait presque 7 fois plus forte que la valeur de référence. On n'a pas décelé la présence de vitellogénine plasmique chez les truites en cage échantillonnées 6 et 18 jours après le traitement, ni celle d'impuretés de la dioxine dans les sédiments après le traitement. On a conclu que les essais en laboratoire sous-estimaient la durée de l'activité de l'OFM dans les conditions *in situ* et que des études supplémentaires devraient porter sur l'évaluation de l'exposition à la formulation au cours des stades vulnérables.

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Introduction

The potential of certain chemicals to affect endocrine systems, and thereby to interfere with reproduction and development in wildlife, is now widely recognized (Ankley et al. 1997). Some of these effects have been attributed to interactions with the aryl hydrocarbon (Ah) receptor, which has been shown to mediate most if not all toxic effects of halogenated aromatic compounds (Okey et al. 1994). Measurements of mixed function oxygenase (MFO) enzyme activity have been used as an indicator of exposure to Ah receptor agonists and as a predictor of potential toxic effects associated with this class of chemicals (Okey 1990). Other effects on endocrine function have been attributed to the ability of compounds to function as hormone mimics through interactions with hormone receptors. Induction of vitellogenin, an estrogen-inducible phosphoprotein normally synthesized in the liver of female oviparous vertebrates during oogenesis, has been used as an indicator of exposure to materials with estrogenic activity (Jobling et al. 1996; Ankley et al. 1997).

Although numerous sources of chemicals with the ability to interfere with endocrine function have been identified in the Great Lakes, recent concern has developed over applications of the lampricide TFM (3-trifluoromethyl-4-nitrophenol). Exposure to field formulations of the lampricide has been shown to elevate MFO activity and affect circulating levels of gonadal sex steroids in fish (Munkittrick et al. 1994). Toxicity identification evaluation (TIE) studies directed by MFO induction have now attributed a portion of the total activity to 3-chloro-nitro-trifluoromethyl-dibenzo-*p*-dioxin formulation impurities (Hewitt et al. 1998*a*, 1998*b*). These isomers have been identified in batches of TFM formulated over the past 10 years at an average total concentration of 280 $mg \cdot L^{-1}$, which translates into loadings of approximately 40 $g \cdot year^{-1}$ to the Great Lakes Basin. Toxic equivalency factors (TEFs) derived in vitro indicate that this class of dioxin congeners are at least as potent inducers of MFO activity as polychlorinated dibenzo-*p*-dioxins (PCDDs) (Hewitt et al. 1998*a*).

Other studies of the effects of formulation components on nontarget species have found that male goldfish (*Carassius auratus*) dosed intraperitoneally with formulation impurities showed significantly lower basal levels of circulating testosterone and 11-ketotestosterone (Hewitt et al. 1996*a*). While compounds directly associated with these disruptions were not identified, further studies have found that fractions of field formulations containing TFM and TFM isomers compete for rainbow trout hepatic estrogen receptors. TFM has been identified as an agonist for the estrogen receptor in vitro as it induced vitellogenin production in primary cultures of rainbow trout hepatocytes (Hewitt et al. 1998*a*).

The ecological significance of MFO induction and disruptions in endocrine homeostasis associated with these laboratory findings is not known. It has not been established whether exposure to TFM and (or) formulation impurities can cause disruptions in endocrine function in vivo during treatments. Furthermore, while short-term static exposures to TFM formulations have shown the potential to elevate MFO activity in the laboratory (Servos et al. 1994; Hewitt et al. 1996*a*), and caged fish are induced during TFM treatment (Munkittrick et al. 1994), the temporal and spatial characteristics of this response following treatment is unknown, as is the persistence of associated dioxin impurities.

The purpose of this study was to investigate potential physiological effects of TFM and its impurities on nontarget fish species following a lampricide treatment. The specific objectives were to determine the temporal and spatial patterns of MFO induction in fish following a lampricide treatment, to determine if induction of vitellogenin occurs in fish after a TFM treatment, and to determine if formulation dioxin impurities are detected after treatment.

Materials and methods

All gases were ultrahigh-purity (Canox, Mississauga, Ont.) grade, solvents were distilled in glass (Caledon Laboratories, Georgetown, Ont.), and chemicals were purchased from Sigma (St. Louis, Mo.) unless otherwise stated.

TFM treatment of Lynde Creek

Lynde Creek drains into the north shore of Lake Ontario and passes through the west end of the town of Whitby, Ont., approximately 30 km east of Toronto (Fig. 1). Lynde Creek has received nine periodic lampricide treatments approximately every 3 years since 1971 (L. Schleen, unpublished data).

The 1995 treatment was initiated on September 22. TFM formulation (batch 1995-2; Hoescht Agrevo, Germany) was applied using drip feeders to upper application points (APs; Fig. 1) on the east and west branches, located 7.88 and 5.60 km above the confluence, respectively. Over 14 h, the east and west branches received 34.26 and 35.65 kg active ingredient, respectively. Approximately 4 h after ceasing the primary applications, a supplemental addition or "boost" of 38.40 kg was applied over 14 h to the east branch immediately above the confluence to coincide with the arrival of the TFM pulse from the west branch (B, Fig. 1). The boost was necessary to ensure the integrity of the pulse of TFM below the confluence of the branches. Treatment concentrations of TFM at sites used for fish caging and collections are provided (Fig. 2). Because of low stream flow the residence time of TFM was extended in the lower reaches of the stream and the sampling times for these sites were adjusted to reflect the transition of the pulse of the lampricide (Fig. 2).

Caged trout exposures and wild fish collections

Two size-classes of juvenile rainbow trout (45.9 ± 1.3 and 3.9 ± 0.3 g (mean ± SD); Rainbow Springs Hatchery, Thamesford, Ont.) were used in caging studies. All fish were obtained 2 weeks prior to exposure and acclimated in laboratory water (13°C, pH 7.5–8.0, hardness 128–133 $mg \cdot L^{-1}$). Fish were fed to satiation until 3 days prior to caging experiments and were not fed during caging. Fish were caged at all sites except those immediately above the confluence (W3, E3). Larger trout were held in wire mesh holding cages and small trout were contained in sealed minnow traps. Trout were caged and (or) collected at a total of eight sites (Fig. 1). Relative to the drip feeder application point on the west branch (AP), a west branch reference site (W1) was located approximately 70 m upstream with a near-exposure site (W2) located approximately 350 m downstream. An east branch reference site (E1) was located approximately 100 m upstream from the application point and was isolated from the lower reaches by a small log dam within an outdoor garden centre. An upper exposure site on the east branch was about 300 m below the application point. Additional sites on each branch were located 30 m above the confluence of the two branches (W3, E3). Two sites 50 m (L1) and 3.2 km (L2) below the confluence were also selected.

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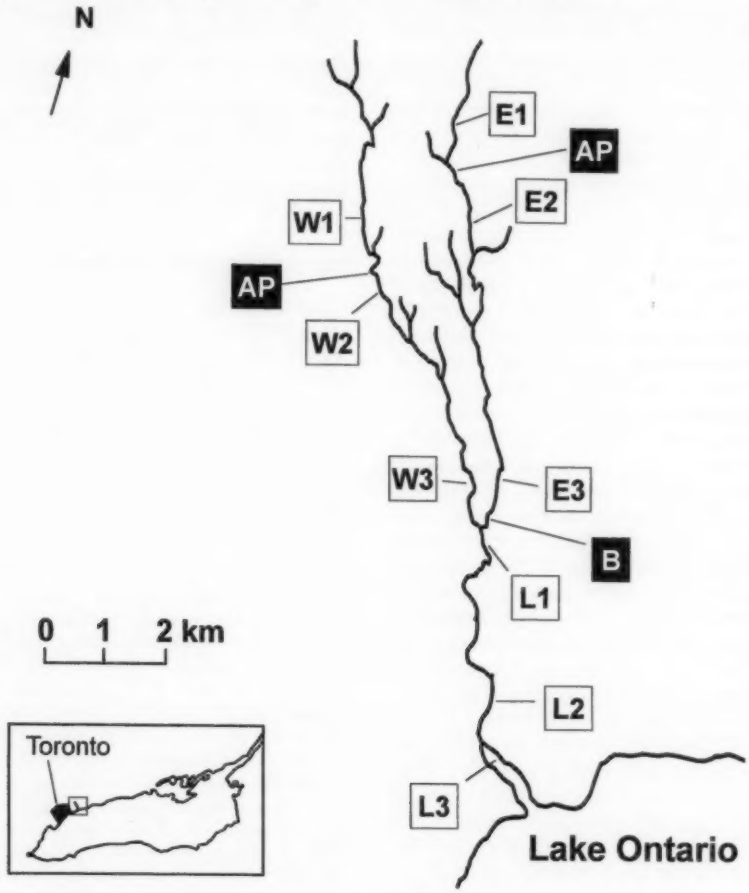
Fig. 1. Map of Lynde Creek system showing site locations of rainbow trout cages, wild fish collections and sediment samples (open boxes), as well as primary (AP) and boost (B) application points of the lampricide (solid boxes).



Small trout were counted and caged in holding tanks at the laboratory the day before placing in the stream. Large trout were transported collectively in a holding cage and randomly distributed to separate holding cages on site. Samples sizes for all studies were 13 smaller trout and 6 larger trout per cage. A 3-day pre-treatment caging study to monitor background levels of MFO induction was conducted 9 days prior to treatment using one cage of each trout size per site. For the treatment study, all caged fish were put in place 1 day before treatment. One cage of small fish and 6 larger trout were sampled 1, 2, 3, 6, and 18 days after transition of the TFM pulse from each site. Twelve white sucker (*Catostomus commersoni*) (37.2 ± 1.5 g) and 12 longnose dace (*Rhinichthys cataractae*) (4.67 ± 0.10 g) were collected 6–8 days prior to treatment at caging locations using a backpack electroshocker unit during pre-treatment caging and 3, 6, and 18 days post-treatment at all sites except L2 where capture efforts were unsuccessful. Further collections were not made at this site.

Relative to the reference sites and sites immediately below the application points, sampling at the W3, E3, and L1 sites was offset by 1 day and the lower L2 site by 2 days (Fig. 2). The cage of larger trout at L2 was lost during the first day of the post-treatment study and MFO activity for small trout only are reported for this site. The larger trout from E1 were lost between day 6 and day 18; therefore, MFO activities at E2 from day 18 were compared to W1. During sampling, pH values ranged from 7.65 to 8.81, conductivity from 0.35 to 0.59 mS, dissolved oxygen from 7.0 to 15.8 $mg \cdot L^{-1}$, and temperatures from 9.6 to 19.0°C in the upper reaches to below the confluence.

All fish were sampled on site. Weights and fork lengths were measured and blood samples from caged trout were collected for vitellogenin analysis. Blood was collected in heparinized tubes and stored on ice until centrifugation for isolation of plasma. Plasma samples were transferred to cryovials and frozen in liquid nitrogen. Fish were euthanized by concussion and spinal severance. Livers were excised, weighed, placed in cryovials, and frozen in liquid nitrogen. Upon return to the laboratory, liver and plasma samples were transferred to storage freezers at –80°C until analysis.

Hepatic MFO induction and plasma vitellogenin analyses

Hepatic MFO induction determined for caged and wild fish as ethoxyresorufin-*O*-deethylase activity (EROD) following the procedure described in van den Heuvel et al. (1995). Liver tissues from smaller caged trout were thawed and homogenized in 1 mL HEPES buffer, whereas subsamples of minced livers from other fish were preweighed and suspended at 1 $g \cdot mL^{-1}$ HEPES. MFO activities were measured as microsomal EROD activity normalized for protein contents in all fish except longnose dace, where enzyme activities were conducted on crude liver homogenates and ex-

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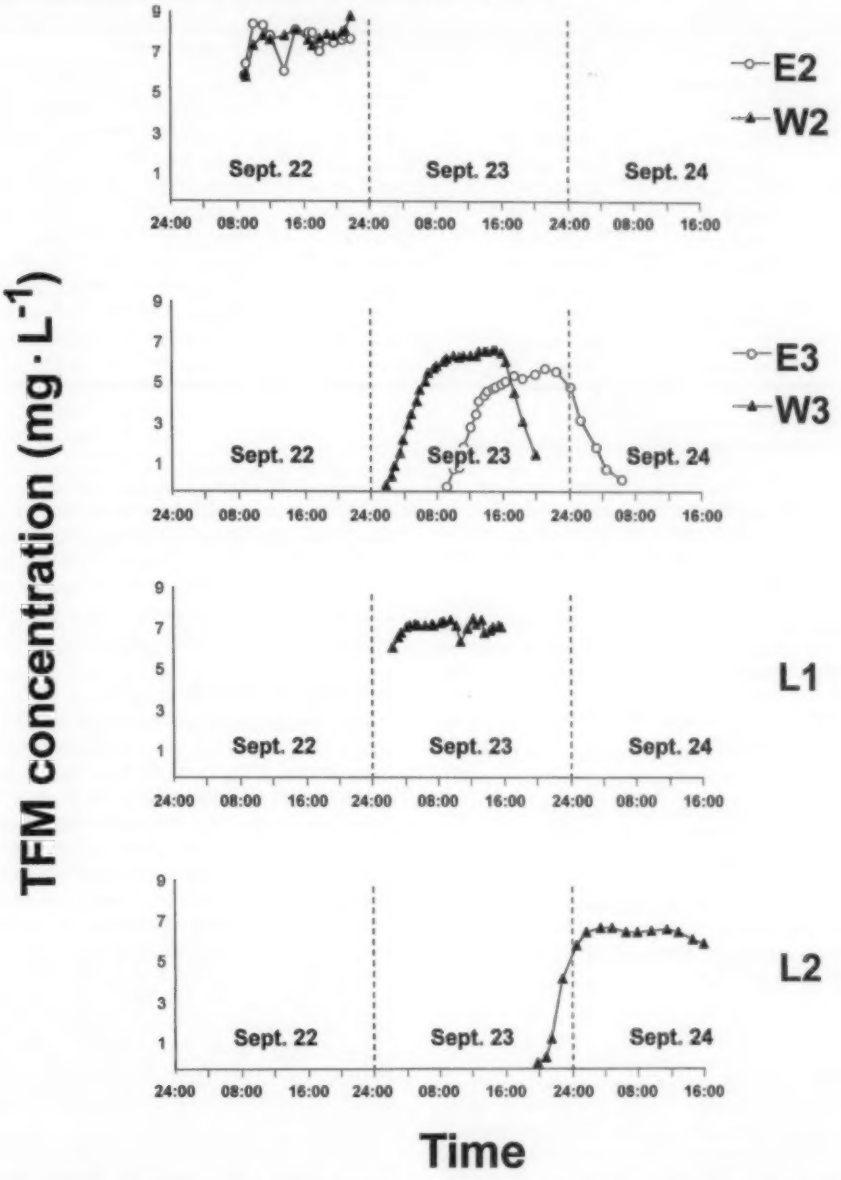
Fig. 2. TFM concentration profiles at designated sites during treatment of Lynde Creek.



pressed as fluorescence units of resorufin produced. EROD activities were determined against a series of external resorufin standards that were prepared daily.

Plasma levels of vitellogenin were analyzed in duplicate using an enzyme-linked immunoassay (ELISA) specific for rainbow trout vitellogenin. Polyclonal rabbit anti-rainbow trout vitellogenin antibody was kindly provided by the late Dr. D.R. Idler (Memorial University, St. John's, NF A1C 5S7). The ELISA was optimized following the protocol developed for goldfish vitellogenin (X. Yao and G.J. Van Der Kraak, unpublished data). Samples were analyzed at dilution factors of 1600, 400, and 40.

Analysis of formulation dioxin impurities

Sediment levels of dioxin impurities were measured to assess the persistence of these compounds from previous treatments and immediately after the 1995 treatment. Sediment cores were collected from three locations within the mouth of Lynde Creek during the pre-treatment study and the top 4 cm were analyzed in 1 cm slices. The top 1 cm of cores were collected from transects of sites W2 and E2 at 1 day and 18 days post-treatment and in the creek mouth 1 day post-treatment. All sediment slices were sealed in glass jars and frozen at –20°C. Frozen core slices were lyophilized, homogenized, and subsampled for their organic carbon contents prior to extraction for formulation impurities.

Approximately 10 g of each slice was spiked with a surrogate 2-trifluoromethyl-3-chloro-7(and 8)-nitro-dibenzo-*p*-dioxin mixture and Soxhlet extracted with dichloromethane overnight. Dichloromethane extracts were reduced under vacuum and brought up to

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Table 1. Hepatic EROD activities determined for caged trout sampled before treatment and 1, 2, 3, 6, and 18 days post TFM treatment at designated sites in Lynde Creek.

		Caged rainbow trout mean hepatic EROD activities (pmol·mg protein^{-1}·min^{-1})					
Site	Size	Pre-treatment	Day 1	Day 2	Day 3	Day 6	Day 18
W1	Small	0.75±0.08	1.69±0.34[b]	0.81±0.13	1.00±0.19	0.62±0.12	0.27±0.07
	Large	1.00±0.16	1.00±0.16	1.35±0.16	1.13±0.19	0.93±0.19	1.57±1.05
W2	Small	0.62±0.05	14.57±2.74	19.64±3.35	14.41±2.44	9.24±2.66	1.86±0.31
	Large	1.02±0.14	32.33±10.67	34.14±7.67	80.75±16.20	6.36±2.06	5.63±2.18
E1	Small	0.62±0.08	1.39±0.32	0.88±0.11	0.76±0.10	4.72±1.23[b]	1.30±0.18
	Large	1.04±0.15	1.38±0.26	1.27±0.42	1.85±0.31	1.06±0.72	na
E2	Small	0.70±0.05	20.16±6.62	21.25±5.56	17.06±2.39	1.00±0.88[a]	7.51±2.43
	Large	1.41±0.15	45.13±9.14	77.26±24.84	15.73±2.33	8.62±1.87	6.62±1.39
L1	Small	0.84±0.08	10.77±3.44	17.73±2.63	8.48±1.77	4.17±2.51[a]	6.56±1.30
	Large	2.80±0.64	84.73±24.68	27.83±6.38	10.58±3.75	3.23±0.87[a]	3.80±0.84[a]
L2	Small	0.93±0.18	7.48±1.30	4.87±0.97	3.92±1.22	0.85±0.16[a]	1.79±1.11

Note: Values are site means ± SE. na, not available.
[a]Not significantly different than reference ($p > 0.05$).
[b]A reference activity induced relative to pre-treatment values at that site ($p < 0.05$).

12 mL in a 1:1 mixture of dichloromethane:hexane and subjected to gel permeation chromatography to remove lipids (ABC Laboratories, Columbia, Mo.). Residual lipids were removed in a micro-alumina cleanup (Huestis and Sergeant 1992). Samples were brought up in a final volume of 20 mL in performance standard for analysis of dioxin impurities by high-resolution gas chromatography – mass spectrometry as described by Hewitt et al. (1996*a*). Concentrations are expressed relative to the content of sediment organic carbon (National Laboratory for Environmental Testing 1996). Additionally, the formulation used for the treatment of Lynde Creek was analyzed in triplicate for chloro-nitro-trifluoromethyl-dibenzo-*p*-dioxin impurities.

Statistics

EROD activites from trout exposures were checked for normality using SYSTAT software (SYSTAT, Inc. 1992) and required log transformation. Two-way analyses of variance (ANOVA) determined significant interactions for each species. One-way ANOVAs were used to determine differences between sites and sampling times. A nested test was used to test for variances within sites. Tukey's HSD pairwise comparisons were used for comparisons of temporal and spatial MFO activities.

Results

Pre-treatment MFO activity

There was no difference in the EROD activities of small trout caged at all sites 9 days prior to the TFM treatment of Lynde Creek ($p > 0.27$). However, relative to fish collected from upstream sites on both branches, low levels of significant ($p < 0.003$) activity were associated with larger trout caged at both sites below the confluence of the east and west branches (L1, L2) (Table 1, L2 data not shown) and with white sucker and longnose dace collected at L1 ($p < 0.02$, Table 2). Significantly larger (fork length, weight: $p < 0.001$) white sucker were collected at L1, and visual identification of sex was possible for 13 of the 48 fish collected. Nine males had gonadosomatic indices (GSI = (gonad weight/body weight) × 100) < 1.0, and MFO activities were pooled with other juvenile fish. The other fish had GSI > 7 and were not included because their maturity may influence MFO activity as observed with other species (Stegeman and Chevion 1980). Since low levels of MFO activity were detected at both sites below the confluence before treatment of TFM, post-treatment determinations of induction at these sites were referenced to pre-treatment values. MFO activity in fish collected at exposure sites on each branch during post-treatment sampling were compared with activity in fish sampled from their respective reference sites above the lampricide application points unless otherwise stated.

Post-treatment MFO activity

The temporal and spatial aspects of MFO induction in caged trout and wild fish sampled after the TFM treatment were similar (Fig. 3). With all fish, the highest levels of induction were found immediately below the application points, at E2 and W2. Induction was rapid and detectable in caged fish at all sites 1 day after transition of the TFM pulse. MFO activity in small trout caged on the west branch peaked at 24-fold on day 2 and had declined to 7-fold by 18 days post-treatment. Induction in larger trout caged in the west branch was higher, being elevated over 30-fold 1 day post-treatment, peaking at over 70-fold by day 3, but declining to 4-fold by day 18 (Fig. 3). The induction with both sizes of fish caged in the east branch of Lynde Creek generally followed these observations. Induction in larger trout peaked at a higher level, but activity in both sizes of caged fish was reduced to approximately fivefold 18 days after treatment (Table 1).

Relative to pre-treatment levels of MFO activity, induction in small trout caged just below the confluence of the two branches closely approximated the temporal observations at the upper exposure sites, while some differences were associated with the pattern of induction in the larger trout sampled at this site. While the 30-fold induction at 1 day post-treatment was nearly identical to the levels observed at W2 and E2, a more rapid decline to pre-treatment levels had occurred after 6 days ($p > 0.95$) (Fig. 3). Small trout caged near the mouth of Lynde Creek (L2) had lower levels of MFO activity than fish caged just below the confluence (Table 1).

Wild white sucker and longnose dace were collected at caging sites and two additional sites on both branches just above the confluence (W3, E3) at 3, 6, and 18 days post-

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Table 2. Hepatic EROD activities determined for wild fish sampled before treatment and 3, 6, and 18 days post-treatment at designated sites in Lynde Creek.

		Wild fish mean hepatic EROD activities			
Site	Species	Pre-treatment	Day 3	Day 6	Day 18
W1	White sucker	2.83±0.38	2.64±0.51	4.58±0.76	25.48±9.56[c]
	Longnose dace	0.81±0.11	1.96±0.35[c]	0.89±0.12	1.26±0.21
W2	White sucker	3.50±0.47	100.32±20.53	147.46±28.97	75.47±18.11
	Longnose dace	0.76±0.11	12.46±1.94	5.90±1.15	6.26±1.46
W3	White sucker	na	20.94±6.98	9.04±1.74[a]	19.65±4.76[b]
	Longnose dace	na	1.19±0.15[a]	2.07±0.31	1.00±0.19[a]
E1	White sucker	4.86±1.16	4.88±0.98	4.76±0.53	12.41±1.47[c]
	Longnose dace	0.88±0.13	1.88±0.20	1.50±0.30	2.43±1.00
E2	White sucker	4.87±0.78	47.41±11.55	35.47±4.66	46.20±11.37[b]
	Longnose dace	0.95±0.15	10.07±1.17	4.42±0.76	2.42±0.46[a]
E3	White sucker	na	20.74±3.23	18.09±4.23[a]	42.34±10.15
	Longnose dace	na	1.21±0.18[a]	2.20±0.53[a]	2.29±0.49[a]
L1	White sucker	14.12±2.15	132.66±18.49	139.46±43.9	85.17±13.24
	Longnose dace	1.45±0.17	1.82±0.70[a]	3.77±0.63	5.67±1.54

Note: Values are site means ± SE. Activities are pmol·mg protein^{-1}·min^{-1} for white sucker and fluorescence units for longnose dace. na, not available.

[a]Not significantly different than reference or pre-treatment ($p > 0.05$).

[b]Not significantly different than reference for that day ($p > 0.05$) but induced relative to pre-treatment ($p < 0.05$).

[c]A reference activity induced relative to pre-treatment values at that site ($p < 0.05$).

treatment. MFO activity in white sucker was higher and more persistent in all cases. Relative to upstream activity, induction was highest in both species at W2, 3 and 6 days post-treatment (Table 2). An apparent decrease in activity is seen for the fish collected from W2 when referenced to W1, but relative induction remains unchanged over time at this site if EROD activities are referenced to pre-treatment values ($p > 0.16$; Table 2). MFO activity in white sucker collected below the application point on the east branch was almost 10-fold on day 3, was 7-fold on day 6, and had declined to approximately 4-fold by day 18 (Fig. 3). Longnose dace had a maximum of fivefold induction on day 3, which had declined to upstream activity by day 18 (Table 2).

Induction was significantly reduced or nonexistent in wild fish collected just above the confluence on each branch (E3, W3). A return to higher levels of MFO activity was found with fish collected just below the confluence of the two branches and below the boost application point of TFM (L1). White sucker collected from L1 were induced approximately 10-fold on day 3 and day 6 and still induced 6-fold by 18 days post-treatment (Fig. 3). Relative to pre-treatment activity, longnose dace collected from this site showed an apparent increase in EROD activity over time to approximately fourfold by day 18 (Table 2).

Small caged trout sampled from both sites above both application points generally had low levels of EROD activity throughout post-treatment sampling periods. Relative to fish sampled at all other times, slight induction (less than fourfold, $p < 0.07$) was associated with upstream fish sampled at day 1 and day 6 from the west and east branches (Table 1). MFO activity in large trout at both upstream sites was consistent with pre-treatment levels ($p > 0.34$). EROD activities in white sucker collected at W1 and E1 were not different than pre-treatment levels ($p > 0.35$) except on day 18 where fish collected at W1 and E1 had 8- and 2.5-fold higher levels, respectively ($p < 0.018$). Induction in longnose dace collected at these sites during pre- and post-treatment sampling was either not significant ($p > 0.05$) or varied by twofold (Table 2).

Caged trout plasma vitellogenin

Plasma from large caged trout collected at all sites on day 18 and from W2 and E2 on day 6 were analyzed for vitellogenin by ELISA. Vitellogenin was not detected (<1.2 mg·mL^{-1}) (Keith et al. 1983) in any of the plasma samples from caged trout sampled 6 and 18 days after treatment at any of the dilutions analyzed.

Persistence of formulation impurities

Formulation impurities were analyzed in sediments collected prior to treatment, and at 1 and 18 days after treatment. The total formulation concentration of chloro-nitro-trifluoromethyl-substituted dioxins was 190 mg·L^{-1}, and these compounds were below detection limits (0.002 ng·g^{-1}) in all sediments analyzed. Method recovery measured using 2-trifluoromethyl-3-chloro-7(and 8)-trifluoromethyl dibenzo-*p*-dioxins was 97 ± 1.4% (mean ± SE).

Discussion

This is one of the first studies evaluating both the physiological effects of TFM treatment on nontarget fish species and the fate of formulation impurities following a lampricide application. This study demonstrated a transitory elevation of hepatic MFO activity in caged trout and two species of wild fish. MFO activity was highest for all fish below the application points and declined downstream to low levels within 18 days. MFO induction was higher and more persistent in wild white sucker than caged trout and may be related to an increased exposure to formulation impurities in

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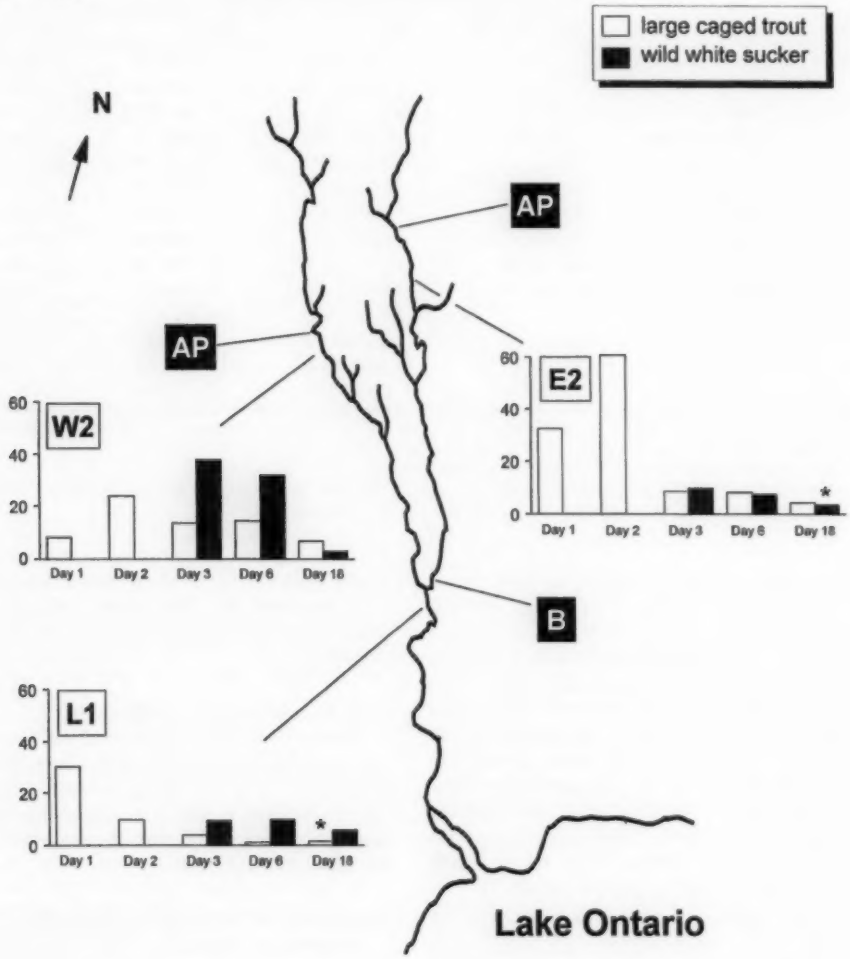
Fig. 3. Post-treatment hepatic MFO induction in large caged trout and wild white sucker at sites exposed to the lampricide TFM. Activities for each site are expressed relative to appropriate reference activities as outlined in text. Asterisks on bars show no significant induction ($p > 0.05$).



wild fish. The transitory effects on MFO activity were consistent with sediment levels of dioxin impurities, which were not detected following treatment. The absence of vitellogenin in caged trout showed that in vitro estrogenic activity associated with TFM was not manifested in vivo following a treatment.

Since TFM was applied separately to both branches of Lynde Creek, two separate nearly identical treatments occurred above the confluence, which provided an opportunity for comparison. In general, the temporal and spatial patterns of MFO activity in caged and wild fish observed in both branches was consistent with treatment data, which show roughly equivalent treatment concentrations of the lampricide was achieved in both branches (Fig. 2). MFO activities in white sucker were consistently higher after treatment than longnose dace and may be due to species-specific contrasts in enzyme activity seen in other species (Pohl et al. 1974). With caged trout, induction was greater in larger fish; however, induction was more persistent in smaller fish from all sites after 18 days (Table 1). Since all trout were immature, these inconsistencies may be related to size and age differences (Thomas et al. 1989).

EROD activities in white sucker were higher and appeared to be more persistent than in caged fish. If MFO activities in white sucker collected on day 18 are compared with reference activities from previous sampling periods, the induction at E2 and W2 is unchanged from day 3 and day 6 ($p > 0.05$). One possible explanation is that the wild fish were exposed to additional quantities of formulation impurities through feeding on invertebrates after exposure. Although stomach contents were not examined, significant invertebrate drift is known to occur following TFM treatments (DuBois and Plaster 1993). More persistent EROD activity was also observed in wild rainbow trout during the 1995 TFM treatment of Bronte Creek with levels returning to pre-treatment levels by 65 days (Servos et al. 1994).

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These results demonstrate that the persistence of MFO induction in some species of resident fish is underestimated by caging studies.

MFO activity in both species of wild fish was lowest in fish collected from E3 and W3. While the magnitude and duration of the TFM pulse was slightly attenuated at these sites during treatment (Fig. 2), the low level of activity indicates a lower level of exposure to the responsible chemicals had occurred at these sites, 5–8 km downstream of the application points. Lower exposures to these chemicals would be expected from partitioning to organic matter in the water column and in sediments. Partitioning would lower downstream exposures to the dioxins as well as other unidentified inducing formulation impurities. Higher levels of EROD activity in white sucker collected 50 m below the confluence at L1 can be attributed to the supplemental boost of TFM applied to the east branch immediately above the confluence (Fig. 2). The concentrations of diphenyl ethers in the mouth of Lynde Creek were about an order of magnitude lower than W2 and E2 on day 18, indicating that a return to a lower exposure had occurred. These observations are consistent with the 1981 treatment of Lynde Creek in which Carey et al. (1988) noted that monochlorinated formulation impurities disappeared from the water column at rates equal to or faster than TFM.

MFO induction in fish sampled from all sites above the confluence were expressed relative to levels for their respective upstream reference sites for a given sampling day. Upstream activities were generally low but some significant fluctuations were evident. The apparent fourfold induction in small trout sampled on day 6 at E1 cannot readily be explained, as large trout sampled at the same time had normal background levels (Table 1). The apparent increase in MFO activity over time with white sucker collected from the reference site on the west branch (Table 2) could be a result of fish movement from downstream as caged trout activities were consistently low (Table 1). Although a small dam just above the application point prevented any movement on the east branch, white sucker MFO activity at E1 on day 18 was threefold over the activities from day 3 and day 6, suggesting an upstream source of unknown contamination.

Detailed studies have attributed a portion of the MFO activity associated with the reference batch from 1990 to three tri-substituted chloro-nitro-trifluoromethyl dibenzo-*p*-dioxin isomers (Hewitt et al. 1998*a*), and these isomers were detected in the batch used for the treatment of Lynde Creek. The induction kinetics in fish exposed to the lampricide treatment are consistent with induction predicted for these compounds. Accumulation of these dioxins and subsequent enzyme induction would be expected to be rapid as these compounds have a maximum predicted log K_{ow} of 4.4 (Hewitt et al. 1996*b*). It has been speculated that a significant level of induction associated with dietary exposure to 2,3,7,8-substituted congeners may arise from a sustained dioxin–Ah receptor complex (Muir et al. 1990). Since it has been established that the tri-substituted dioxin impurities present in TFM formulations are not substituted exclusively in the 2,3,7 positions (Hewitt et al. 1998*a*) a rapid decline of MFO activity would be expected after cessation of exposure. Substitution in non-2,3,7 positions has been shown to facilitate the rapid metabolism of these compounds by MFO enzymes (Okey 1990), and the low bioaccumulation of di- and tri-chlorinated dioxins in fish have been attributed to excretion of these compounds (Opperhuizen and Sijm 1990).

The absence of vitellogenin in plasma of caged trout exposed to the TFM treatment is likely due to a combination of the weak estrogenic activity of TFM and a transient exposure. In the treatment of Lynde Creek, the hydraulic residence time of the TFM pulse ranged from 14 h in upper reaches to 16 h in the mouth (Fig. 2). The persistence of TFM in the water column after treatments is relatively short, a half-life of 3–5 days was estimated for a previous treatment of Lynde Creek (Carey et al. 1988). During exposure, TFM is rapidly accumulated, conjugated with glucuronic acid in the liver and kidney and excreted via the biliary fecal route in fish (Kane et al. 1994). After cessation of exposure, depuration is also rapid, clearance from all tissues usually occurs within 24 h. The short exposure, rapid clearance, and the weak affinity of 5.03×10^{-5} of TFM for hepatic rainbow trout estrogen receptors relative to estradiol (Hewitt et al. 1998*b*) all would contribute to the lack of a detectable response. While the results of this study indicate that in vitro estrogenic activity is not manifested in vivo following field treatments of the lampricide, further studies using pure TFM are required to confirm whether it functions as an estrogen receptor agonist in vivo.

This study has shown that responses mediated by the estrogen receptor and the Ah receptor in fish were either not observed or were transitory after treatment with the lampricide TFM. It is not currently possible to make a definitive conclusion regarding the ecological significance of TFM treatments to nontarget resident fish species in streams within the Great Lakes Basin. Although MFO induction is widely used as an indicator of exposure to Ah receptor agonists, the relevance of Ah receptor genetic products expressed at low levels to physiological performance is not fully understood. MFO activities measured in three species of fish following treatment indicate that exposures to dioxin impurities as well as unidentified inducing formulation impurities are transient and restricted to treatment zones. One question that arises from these findings that was not addressed in this study is whether exposure to field formulations can affect development of aquatic species, particularly during sensitive developmental stages. The lampricide treatment season spans from the late spring through to the early fall, encompassing critical developmental stages of several species. For example, it has been documented that adult fish are more sensitive to the acute toxicity of TFM during spawning (National Research Council of Canada 1985). Given the presence of MFO induction after treatment and the documented effects on early life stages caused by chemicals that interact with the Ah receptor (Walker and Peterson 1991), an examination of effects during periods such as spawning and early life stages is now needed to achieve a better understanding of the ecological effects associated with lampricide treatments.

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Synergistic effects of nutrient loading and omnivorous fish on phytoplankton biomass

Ray W. Drenner, Kirsten L. Gallo, Robert M. Baca, and J. Durward Smith

Abstract: To examine how nutrient loading interacts with omnivorous fish to regulate the biomass of phytoplankton of lakes, we conducted a 1-month summer mesocosm experiment and a 1.5-year pond experiment. For the mesocosm experiment, we used a 2 × 2 × 2 factorial design in which two levels of nutrient loading were cross-classified with two levels of omnivorous fish and two levels of clay. We found a significant nutrient–fish interaction effect for chlorophyll biomass with the effects of fish being greater in the presence of nutrient loading. Clay did not affect the nutrient–fish interaction effect for chlorophyll. In the pond experiment, we used a 2 × 2 factorial design in which two levels of nutrient loading were cross-classified with two levels of omnivorous fish. We did not detect a significant nutrient–fish interaction effect for chlorophyll except at the end of the second summer of the experiment when the biomass of omnivorous fish was high and the cumulative effects of nutrient loading were greatest. Chlorophyll was suppressed by dense populations of Eurasian watermilfoil (*Myriophyllum spicatum*), which developed in some ponds during the experiment. The role of the nutrient–fish synergism in regulating phytoplankton biomass may be reduced in lakes with Eurasian watermilfoil.

Résumé : Nous avons réalisé une expérience en mésocosme d'une durée d'un mois en été et une expérience en étangs de 1,5 an afin d'étudier les effets des interactions entre la charge en matières nutritives et les poissons omnivores sur la biomasse du phytoplancton des lacs. Pour l'expérience en mésocosme, nous avons utilisé une configuration factorielle 2 × 2 × 2 dans laquelle deux niveaux de charge en matières nutritives ont pfait l'objet d'une classification combinée avec deux niveaux de poissons omnivores et deux niveaux d'argile. Nous avons trouvé une interaction matières nutritives–poissons significative pour la biomasse de chlorophylle, l'effet des poissons s'avérant plus important en présence d'une charge de matières nutritives. L'argile n'a pas influé sur l'effet de l'interaction matières nutritives–poissons sur la chlorophylle. Pour l'expérience en étangs, nous avons utilisé un configuration factorielle 2 × 2 dans laquelle deux niveaux de matières nutritives ont fait l'objet d'une classification combinée avec deux niveaux de poissons omnivores. Nous n'avons pas décelé d'effet significatif de l'interaction matières nutritives–poissons pour la chlorophylle sauf à la fin du deuxième été de l'expérience lorsque la biomasse des poissons omnivores était élevée et que les effets cumulatifs de la charge en matières nutritives étaient les plus importants. La chlorophylle a été éliminée par des populations denses de myriophille en épi (*Myriophyllum spicatum*) qui sont apparues dans certains étangs pendant l'expérience. Le rôle de la synergie matières nutritives–poissons dans la régulation de la biomasse phytoplanctonique pourrait être réduit dans les lacs ou se trouve cette myriophille.

[Traduit par la Rédaction]

Introduction

Phytoplankton productivity and biomass are regulated simultaneously by both consumers and nutrients such as phosphorus and nitrogen (McQueen et al. 1986; Northcote 1988; McCauley et al. 1989; Elser et al. 1990). Although ecologists usually study the effects of consumers and nutrients without regard to their combined effects, consumers and nutrients may operate interdependently to regulate lake systems. For example, recent studies of *Rana utricularia* tadpoles have suggested that differences in consumers in pond food webs can dramatically alter how organisms respond to nutrients that regulate primary productivity (Leibold and Wilbur 1992). One significant implication of Leibold and Wilbur's (1992) study is that the combined effects of nutrients and consumers on primary producers cannot always be predicted as the sum of their separate effects.

Most of the previous research on nutrient–fish interactions and their effects on phytoplankton productivity and biomass has focused on visually feeding zooplanktivorous fish. McQueen et al. (1986) hypothesized that the enhancement of phytoplankton by zooplanktivorous fish diminished with increased eutrophication. While some field studies have been consistent with this hypothesis (Post and McQueen 1987; McQueen et al. 1989; Lafontaine and McQueen 1991), mesocosm experiments with visually feeding fish have not detected the hypothesized nutrient–fish interaction effect for phytoplankton biomass (Drenner et al. 1989, 1990; Lancaster and Drenner 1990). Based on reviews of published experiments, Lancaster and Drenner (1990) and DeMelo et al.

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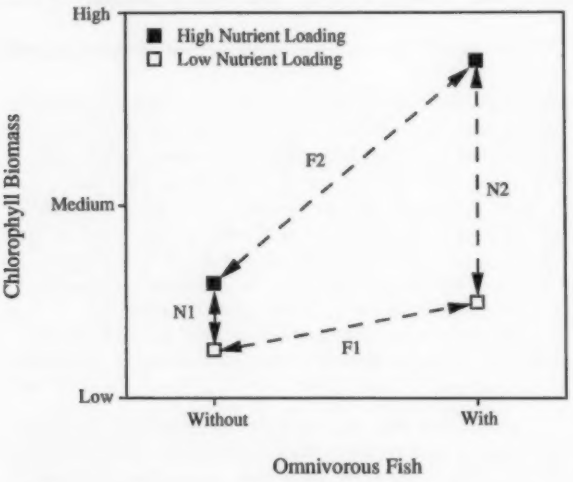
Fig. 1. Hypothesized synergistic effects of nutrient loading and omnivorous fish on chlorophyll. In this interaction, omnivorous fish have a greater effect (magnitude of effect shown by F2) at high nutrient loading than at low nutrient loading (F1), while nutrient loading has a greater effect (N2) with omnivorous fish than without (N1).



(1992) concluded that there is little evidence supporting the hypothesis that the impacts of zooplanktivorous fish may be stronger in oligotrophic systems than in eutrophic systems.

Compared with visually feeding zooplanktivorous fish, there has been little research on how nutrients and omnivorous fish may interact to affect the water quality of lakes. Omnivorous fish dominate the fish biomass of lakes worldwide (Stenson et al. 1978; Miranda 1983; Meijer et al. 1990; Riemann et al. 1990) and affect the productivity and biomass of phytoplankton (review in Drenner et al. 1996). Although omnivorous fish can affect phytoplankton directly by selectively consuming larger phytoplankton species, the most commonly observed effect of omnivorous fish has been indirect enhancement of phytoplankton productivity and biomass (review in Drenner et al. 1996). Omnivorous fish enhance phytoplankton either by suppressing herbivorous zooplankton, resuspending settled phytoplankton or by acting as "nutrient pumps" when they consume benthic nutrients in the form of periphyton and sedimented detritus and then excrete the nutrients in dissolved form into the water column (Lamarra 1975; Brabrand et al. 1990; Mather et al. 1995; Schaus et al. 1997).

Drenner et al. (1996) examined the separate and combined effects of nutrients and an omnivorous clupeid, gizzard shad (*Dorosoma cepedianum*), on phytoplankton productivity and chlorophyll biomass in a tank-mesocosm experiment of factorial design. Five biomass levels of gizzard shad were cross-classified with two levels of lake trophic state achieved by filling mesocosms with water and plankton transported by truck from an oligotrophic and a eutrophic lake. To sustain the differences in trophic state, mesocosms with water from the eutrophic lake received higher additions of nutrients (phosphorus (P) + nitrogen (N)) than mesocosms with water from the oligotrophic lake. Nutrient loading had a greater enhancement effect on chlorophyll biomass in mesocosms with high biomasses of gizzard shad than low biomasses of gizzard shad. Gizzard shad had a greater enhancement effect on chlorophyll biomass in the eutrophic mesocosm systems than the oligotrophic systems. Based on these results, Drenner et al. (1996) hypothesized that the impacts of nutrient loading may interact synergistically with omnivorous fish to increase phytoplankton biomass (Fig. 1), an effect opposite that proposed for zooplanktivorous fish by McQueen et al. (1986). We here present the results of a mesocosm experiment and a pond experiment testing the hypothesized nutrient–fish synergism.

Methods

Mesocosm experiments

The mesocosm experiment was conducted in white fiberglass tank-mesocosms at Texas Christian University, Fort Worth. Mesocosms were 1.8 m in diameter, 2.2 m high, and contained about 5500 L. Mesocosms were filled with water pumped from a shallow (2.5-m maximum depth), 0.7-ha pond adjacent to the tank facility. The pond is eutrophic with concentrations of total phosphorus (TP) and total nitrogen (TN) ranging from 30 to 250 μg/L and from 400 to 3850 μg/L, respectively. To prevent stratification and simulate the wind-induced mixing of shallow lake systems, each mesocosm was continually mixed with an airlift mixer system which entrained water from near the bottom of the mesocosm and injected it across the surface of the mesocosm (Drenner et al. 1986).

The objective of the mesocosm experiment was to examine the separate and combined effects of nutrient loading and omnivorous fish on the biomass of phytoplankton and test whether these effects were impacted by the presence of clay. We included clay as a treatment factor to examine if clay resuspension by feeding activities of omnivorous fish could affect the interrelationship between nutrient loading and fish. Suspended clay can alter plankton community responses to nutrient loading (Cuker 1993). We used 16 mesocosms in a factorial design in which two levels of nutrient addition (with and without P + N) were cross-classified with two levels of omnivorous fish (with and without blue tilapia + carp) and two levels of clay (with and without clay). Each of the eight treatment combinations were replicated twice.

Mesocosms were filled on September 18, 1992, and stocked with fish on September 24 (Table 1). For omnivorous fish, we used blue tilapia (*Tilapia aurea*) combined with carp (*Cyprinus carpio*). Blue tilapia feed on zooplankton, phytoplankton, periphyton, and detritus (Spataru and Zorn 1978; Vinyard et al. 1988; Drenner et al. 1997). Carp consume zooplankton when young and feed as adults on zooplankton, detritus, and benthic invertebrates (Gurzeda 1965; Szumiec 1966; Opuszynski 1981; Richardson et al. 1990). With the exception of a study by Losos and Hetesa (1973) examining the effects of carp fry and fertilization, no studies have examined how the ecological effects of carp and tilapia may change with nutrient loading. During the night of September 24, some carp jumped out of the mesocosms and died. Because we were uncertain as to which mesocosms lost carp, we stocked two additional carp into each mesocosm on September 25 and placed netting over the top of each mesocosm to keep the carp in the mesocosms.

For nutrients, we added P combined with N. Phosphorus has been generally considered to be the primary nutrient limiting phytoplankton growth, but based on a review of experimental studies of nutrient effects on primary productivity, Elser et al. (1990) concluded that combined P + N enrichment enhanced algal growth more substantially than did addition of N or P singly. To each of the high nutrient mesocosms we added 3.2 g of KH_2PO_4 and 11.8 g of NH_4Cl every 2 or 3 days beginning on September 24 for loading rates of 1.3 kg $P \cdot ha^{-1} \cdot day^{-1}$ and 4.4 kg $N \cdot ha^{-1} \cdot day^{-1}$. Clay was

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Table 1. Range of numbers and average biomass of fish stocked and recovered in the mesocosms containing treatments with fish.

Nutrient and clay treatment	Fish treatment	No. stocked	Biomass stocked (g)	No. recovered	Biomass recovered (g)
Without nutrients, without clay	Tilapia	21–22	221	21–22	282
	Carp	2–3	532	2–3	590
With nutrients, without clay	Tilapia	24–34	195	24–31	321
	Carp	2–3	555	3–4	847
Without nutrients, with clay	Tilapia	16–23	275	12–21	243
	Carp	2–6	512	3–7	785
With nutrients, with clay	Tilapia	24–25	210	24	268
	Carp	2	564	2–3	684

Note: Some tanks contained fish fry.

added as 100 g of sodium montmorillonite (Wyoming bentonite) per mesocosm every 2 or 3 days beginning on September 24. Mesocosms were sampled on days 8 and 15 after fish stocking.

Mesocosms were drained and fish recovered on October 24 (Table 1). All tilapia and carp survived except for the carp that jumped out of the mesocosms on September 24. Because supplemental carp were added after the start of the experiment, the difference in stocked and recovered weight of carp does not indicate growth.

In the mesocosm experiment, integrated water-column samples for analyses of TP, TN, chlorophyll *a*, and turbidity were collected with a PVC tube lowered to near the mesocosm bottom. Samples for TP were digested with potassium persulfate (Menzel and Corwin 1965) and analyzed using a modification of the malachite green method (Van Veldhoven and Mannaerts 1987) in which 1 mL of color reagent was added to 5-mL subsamples of the 20-mL total digestions and absorbance measured at 610 nm. Samples for TN were digested with alkaline potassium persulfate (D'Elia et al. 1977), and absorbance was measured at 220 nm (APHA 1985). Chlorophyll *a* samples on all dates were filtered through Millipore HAWP membrane filters (0.45-μm pore size); the filters were wrapped in aluminum foil and frozen. Chlorophyll *a* was extracted in 2:1 chloroform:methanol solution in the dark at 20–22°C for at least 4 h, and absorbance was read at 665 nm (Wood 1985). Turbidity was analyzed using a Hach turbidimeter.

Gross phytoplankton primary productivity was determined by the oxygen method (Lind 1974) with water siphoned from the mesocosms into two 250-mL light bottles and one 250-mL dark bottle and incubated for 24 h. Because the airlift mixers prevented the tanks from stratifying and continually cycled the phytoplankton into light at the surface of the tanks, we incubated bottles at approximately 2 cm below the surface only. Although measurement of the primary production only at the surface may have limited any potential compensation by light limitation in the high nutrient treatments, we feel that our approach is reliable as an estimate of surface primary production. Dissolved oxygen was measured with a YSI Model 57 oxygen meter.

Pond experiment

The objective of the pond experiment was to examine the separate and combined effects of nutrient loading and omnivorous fish on the water quality and biotic communities of larger scale and more environmentally complex pond systems. We used a 2 × 2 factorial design in which two levels of nutrient loading (with and without P + N) were cross-classified with two levels of omnivorous fish (with and without carp). The experiment was conducted in 23 earthen ponds at the Eagle Mountain Fish Hatchery, Fort Worth. The treatment combination with nutrients added and omnivorous fish present was replicated five times, while the other three treatment combinations were replicated six times. Ponds ranged from 0.15 to 0.66 ha and had an average depth of 0.6 m (maximum depth 1.2 m). Ponds were filled with water from Eagle Mountain Lake, a eutrophic reservoir.

As the omnivorous fish treatment, we used carp, one of the major species of omnivorous fish in U.S. reservoirs (Miranda 1983). Ponds were stocked with fish from October 1991 through February 1992. Eleven of the 23 ponds were stocked with approximately 1200 fingerling carp/ha. Carp ponds were also stocked with adult gizzard shad, but they became established at low density in only one pond. All ponds were stocked with fingerling largemouth bass (*Micropterus salmoides*) (120/ha) and bluegill (*Lepomis macrochirus*) (1200/ha) according to guidelines of the Texas Chapter of the American Fisheries Society (1986). We believe that including these centrarchids in the ponds results in a more realistic assessment of carp effects, because lakes with carp usually contain largemouth bass and bluegill (Miranda 1983), and these centrarchids affect the phytoplankton and water quality of lakes through their effects on zooplankton and nutrient concentrations (Hambright et al. 1986; Carpenter et al. 1987; Mittelbach et al. 1995). We also stocked all ponds (25/ha) with sterile triploid grass carp (*Ctenopharyngodon idella*), which feed on aquatic vegetation (Kirk 1992, Cassani et al. 1995), but poor survival of grass carp prevented any apparent effect of them on macrophyte abundance.

To manipulate nutrient loading, we added liquid agricultural fertilizer (10:34:0, N:P:K) into 11 of the ponds. We selected this fertilizer because it had been used in previous experiments to stimulate phytoplankton productivity of the ponds (B. Farquhar, Texas Parks and Wildlife Department, Austin, personal communication). We applied diluted fertilizer across the water surface (Metzger and Boyd 1980) at a rate of 9.4 L fertilizer/ha. During the first spring and summer, fertilizer was added to the ponds every 2 weeks from March 20 – May 29, 1992, for loading rates of 0.29 kg $P \cdot ha^{-1} \cdot day^{-1}$ and 0.09 kg $N \cdot ha^{-1} \cdot day^{-1}$. During the second spring and summer, fertilizer was added to the ponds every 2 weeks from March 18 – June 25, 1993, and monthly in July and August 1993 for loading rates of 0.21 kg $P \cdot ha^{-1} \cdot day^{-1}$ and 0.06 kg $N \cdot ha^{-1} \cdot day^{-1}$.

From March 1992 through August 1993, ponds were sampled monthly to assess TP, TN, chlorophyll *a*, and turbidity. Integrated water-column samples for water quality analyses were taken with a PVC tube from the top 0.5 m near the deepest area of the pond. Samples were analyzed using the same techniques as for the mesocosm experiments.

From July 30 to August 16, 1993, we sampled each pond to estimate biomass of macrophyte species. Twelve samples were taken per pond along a line transect running from near the water inflow area to the opposite side of the pond. The first sample was taken 0.5 m offshore, and the other 11 samples were taken at randomly selected distances along the transect. At each site, all vegetation above the sediment surface was removed from a 0.023-m^2 area (15 × 15 cm). Macrophytes were placed in nylon-mesh bags and

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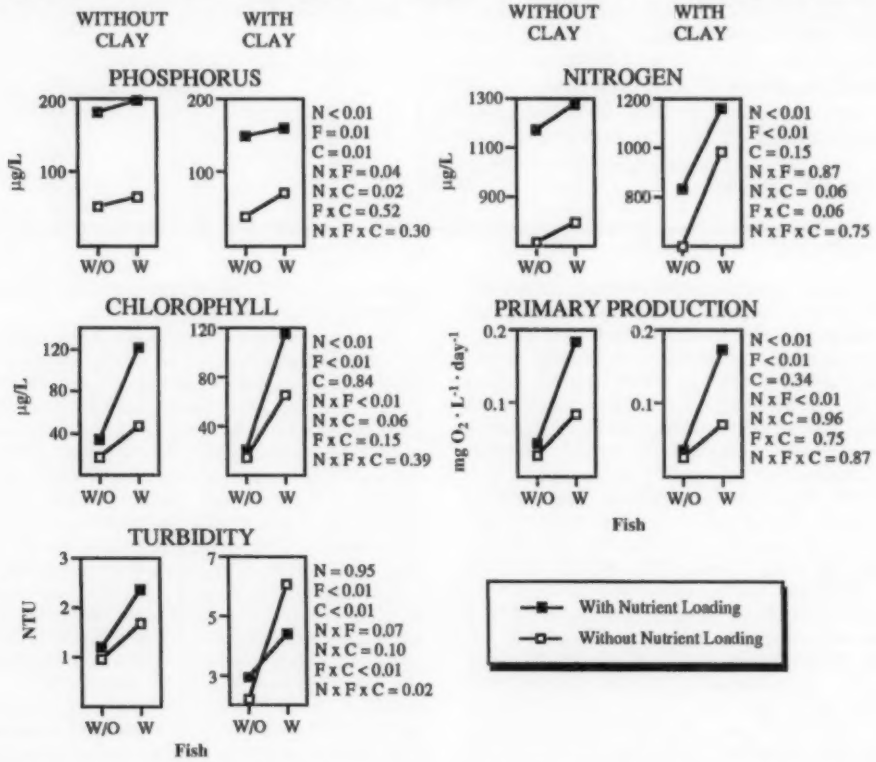
Fig. 2. Mean responses of water quality variables to nutrients, omnivorous fish (tilapia + carp), and clay manipulations of the mesocosm experiment. Each point represents the mean of the two sampling dates for the replicate mesocosms within the treatment combination. Probability values from ANOVA of nutrient loading effects (N), omnivorous fish effects (F), clay effects (C), and their interaction effects are indicated to the right of each graph. W, With omnivorous fish, W/O, without omnivorous fish.



stored in water until they were sorted to species. Specimens were blotted with paper towels, dried in the sun, and weighed.

Densities of macrozooplankton were sampled August 20, 1993, with one zooplankton tow collected near the deepest area of each pond using an 80-µm Wisconsin plankton net. Zooplankton were preserved in 10% sugar–formalin and counted with a Wild stereomicroscope.

To assess fish populations, we electrofished all ponds by boat on either October 29 or November 13, 1993, during the daytime. The electrofishing unit consisted of a 3750-W generator and a 10-amp pulsator. Electrofishing was conducted along the entire perimeter of each pond for approximately 10 min. Fish were held in stock tanks until weights were taken, then they were returned to the pond.

The data sets were analyzed by ANOVA for cross-classified factors using SYSTAT version 5.2.1 (Wilkinson 1992). The analysis allowed us to examine main effects and interaction effects. Main effects reflect the separate effect of each treatment factor (nutrient loading, omnivorous fish, or clay), while interaction effects reflect any interdependence between effects of nutrient loading, omnivorous fish, and clay on response variables. For response variables having significant interaction effects, we tested for simple effects or the effects of nutrient loading, omnivorous fish, or clay at each level of the other treatment factor. Because of the short duration of the mesocosm experiment and minimal community seasonal succession expected, we were not interested in examining time-related effects, and they are not considered in the analyses. For the pond experiment, we analyzed for main and interaction effects of nutrient loading and omnivorous fish (carp) over the course of the 1.5-year experiment and on each monthly sampling date. Because of low replication and statistical power, we chose a probability level of alpha < 0.10 to reduce the chance of making the type II error of failing to reject a false null hypothesis.

Results

Mesocosm experiment

In the mesocosm experiment, nutrient loading significantly increased TP, TN, primary productivity, and chlorophyll (Fig. 2). The presence of omnivorous fish significantly increased TP, TN, primary productivity, chlorophyll, and turbidity. The presence of clay significantly decreased TP and significantly increased turbidity. We detected nutrient–fish interaction effects for TP, primary productivity, chlorophyll, and turbidity. Analysis of simple effects showed that nutrient loading increased TP in mesocosms with and without omnivorous fish ($P < 0.001$), while omnivorous fish affected TP in mesocosms without nutrient addition ($P < 0.01$) but not TP in mesocosms with nutrient addition ($P > 0.10$). Nutrient effects on primary productivity were greater in the mesocosms with omnivorous fish ($P < 0.001$) compared with mesocosms without omnivorous fish ($P < 0.10$). Effects of omnivorous fish on primary productivity were greatest in the mesocosms with nutrients added ($P < 0.001$) compared with

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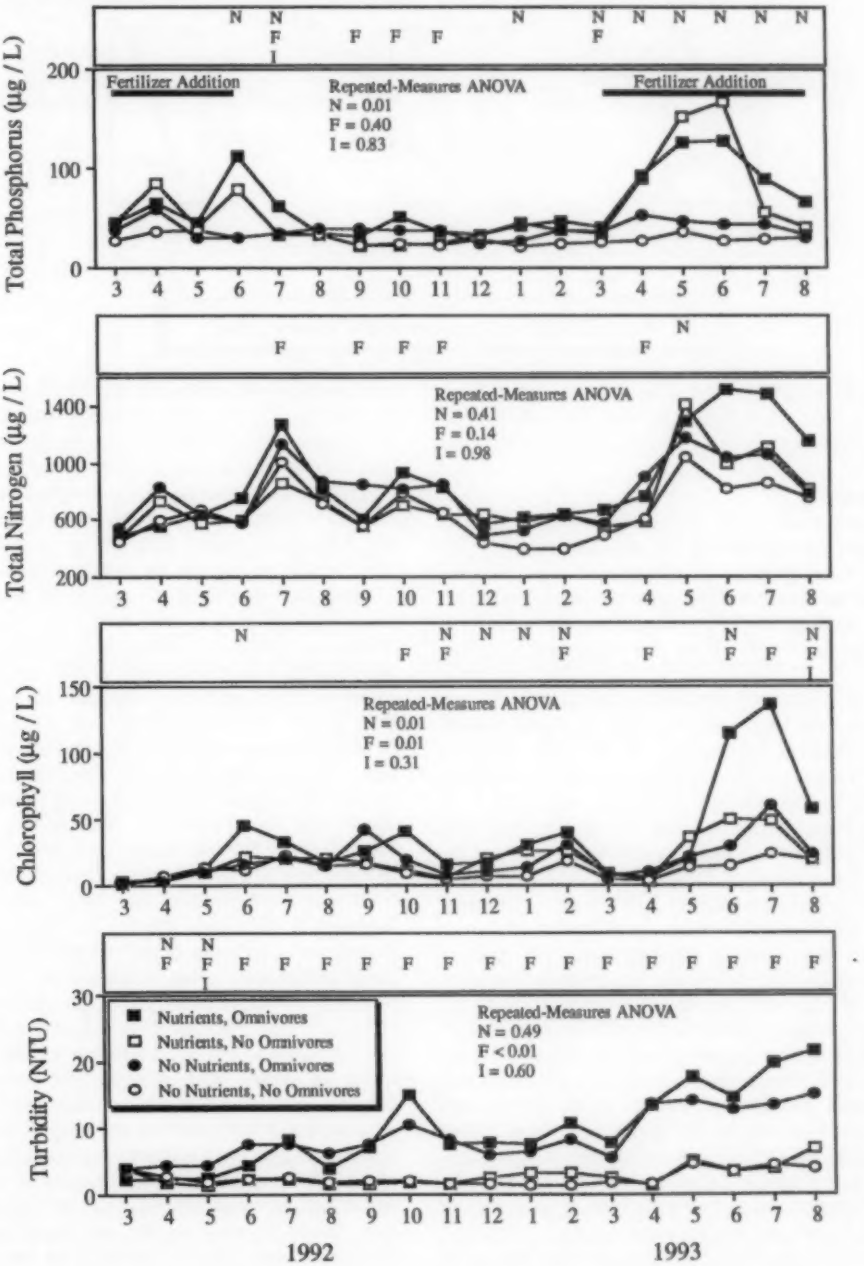
Fig. 3. Mean responses of water quality variables of the ponds to combinations of nutrient loading and omnivorous fish (carp) for the 18 monthly sampling dates. Each point represents the mean of all sampling dates for the replicate ponds within the treatment combination. Results of repeated-measures ANOVA for nutrient loading effects (N), omnivorous fish effects (F), and their interaction effects (I) are presented inside each panel. Results of ANOVA for nutrient loading effects (N), omnivorous fish effects (F), and their interaction effects (I) on each sampling date are presented above each panel. See bottom panel for key.



the mesocosms without nutrients ($P < 0.10$). Nutrient effects on chlorophyll were greatest in the mesocosms with omnivorous fish ($P < 0.001$) and not significant in mesocosms without omnivorous fish ($P > 0.10$). While omnivorous fish effects on chlorophyll were significant in mesocosms with and without nutrients added ($P < 0.001$), the magnitude of the fish effects were greatest in the mesocosms with nutrients added compared to the mesocosms without nutrients

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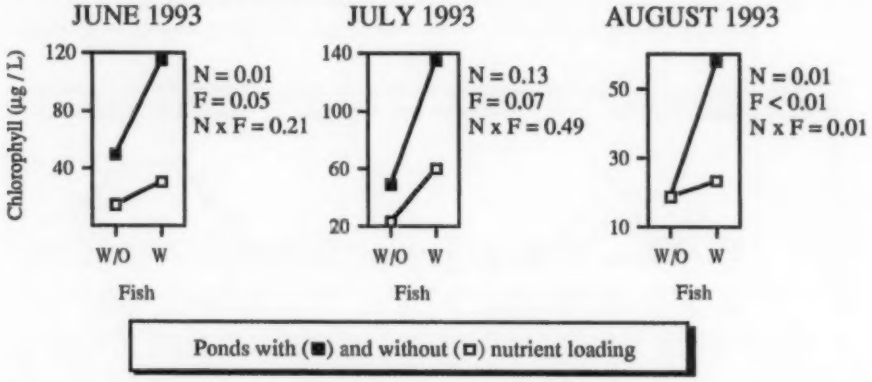
Fig. 4. Mean responses of chlorophyll of the ponds to combinations of nutrient loading and omnivorous fish (carp) during June, July, and August 1993. Each point represents the mean of the replicate ponds within the treatment combination. Probability values from ANOVA of nutrient loading effects (N), omnivorous fish effects (F), and their interaction effects (N × F) are indicated to the right of each graph. W, With omnivorous fish; W/O, without omnivorous fish.



added. The nutrient–fish synergism for primary productivity and chlorophyll was not affected by clay as indicated by nonsignificant nutrient–fish–clay interaction effects (Fig. 2).

Pond experiment

Over the course of the 1.5-year pond experiment, nutrient loading had significant effects on TP and chlorophyll, while carp had significant effects on chlorophyll and turbidity (Fig. 3). We did not detect a significant interaction effect between nutrient loading and carp, suggesting the effects of nutrient loading and omnivorous fish operated independently.

Analysis of the effects of nutrient loading, carp and their interaction effects on each sampling date revealed significant effects of nutrient loading for TP on nine sampling dates but for TN on only one sampling date (Fig. 3). While nutrient loading had significant effects on chlorophyll on seven sampling dates, nutrient loading had significant effects on turbidity on only two sampling dates at the start of the experiment. Concentrations of TP and TN were significantly greater in ponds containing carp on 5 of 18 sampling dates. Concentrations of chlorophyll were significantly greater in ponds containing carp on seven sampling dates, with most of these dates during the last year of the experiment. The most intense effects of carp were detected for turbidity with turbidity being significantly greater in the presence of carp on all dates except the initial sampling date. In general, the enhancement of turbidity in the presence of carp increased during the experiment, probably as a result of the growth and increase in biomass of carp in the ponds. The time-related trend toward increased turbidity in the presence of carp diminished during the winter months, probably because declines in water temperature reduced the feeding activities of the carp. In the second summer we observed carp feeding in very shallow water, expelling turbidity plumes from their opercular cavities as they fed. Such behavior was not observed during cold periods of the year.

During the fertilization period in the first year of the pond experiment (1992), nutrient–fish interaction effects on chlorophyll were not significant on any sampling date (Fig. 3). It was not until June, July, and August of the second fertilization period (1993) that we observed greater effects of carp on chlorophyll in fertilized ponds, but the nutrient–fish interaction effects were only significant in August (Fig. 4). Analysis of simple effects revealed that carp enhanced chlorophyll in the presence of nutrient loading ($P < 0.001$) and not in the ponds without nutrient loading ($P > 0.25$). Nutrient loading enhanced chlorophyll in the presence of carp ($P < 0.001$) and not in the ponds without carp ($P > 0.25$).

The ponds contained 11 species of macrophytes with the abundant species including American lotus (*Nelumbo lutea*), American pondweed (*Potamogeton nodosus*), bushy pondweed (*Najas guadalupensis*), and Eurasian watermilfoil (*Myriophyllum spicatum*). Species occurring in fewer than five ponds included *Chara* sp., narrow-leaf pondweed (*Potamogeton obtusifolius*), smartweed (*Polygonum amphibium*), and coontail (*Ceratophyllum demersum*). Carp significantly reduced the biomass of bushy pondweed, filamentous algae, and the number of macrophyte species per pond (Fig. 5). We did not detect significant effects of nutrient loading or nutrient–fish interaction effects on macrophytes.

Eurasian watermilfoil may have affected water quality variables in the ponds. During the experiment, we observed a decrease in turbidity in the ponds with carp as watermilfoil began to colonize some of the ponds. To examine the relationship of watermilfoil and water quality, we plotted turbidity, TP, and chlorophyll in August as functions of watermilfoil biomass (Fig. 6). Turbidity, TP, and chlorophyll were lower in ponds with watermilfoil biomass greater than 1 kg/m^2. Using an analysis of covariance with watermilfoil as a covariate with nutrient and carp as factors, we detected significant effects of nutrient loading ($P < 0.009$) and carp ($P < 0.001$), a nutrient–fish interaction effect ($P < 0.002$) and effects of watermilfoil ($P < 0.096$) on chlorophyll in August. Based on these results, we hypothesize that nutrient–fish synergisms may be regulated by the presence of dense populations of Eurasian watermilfoil.

In August 1993, nutrient loading decreased densities of copepodids, while densities of cladocerans (primarily *Bosmina* sp. and *Diaphanosoma* sp.) and rotifers were significantly higher in ponds containing carp (Fig. 5). The biomasses of bluegill and largemouth bass collected by elec-

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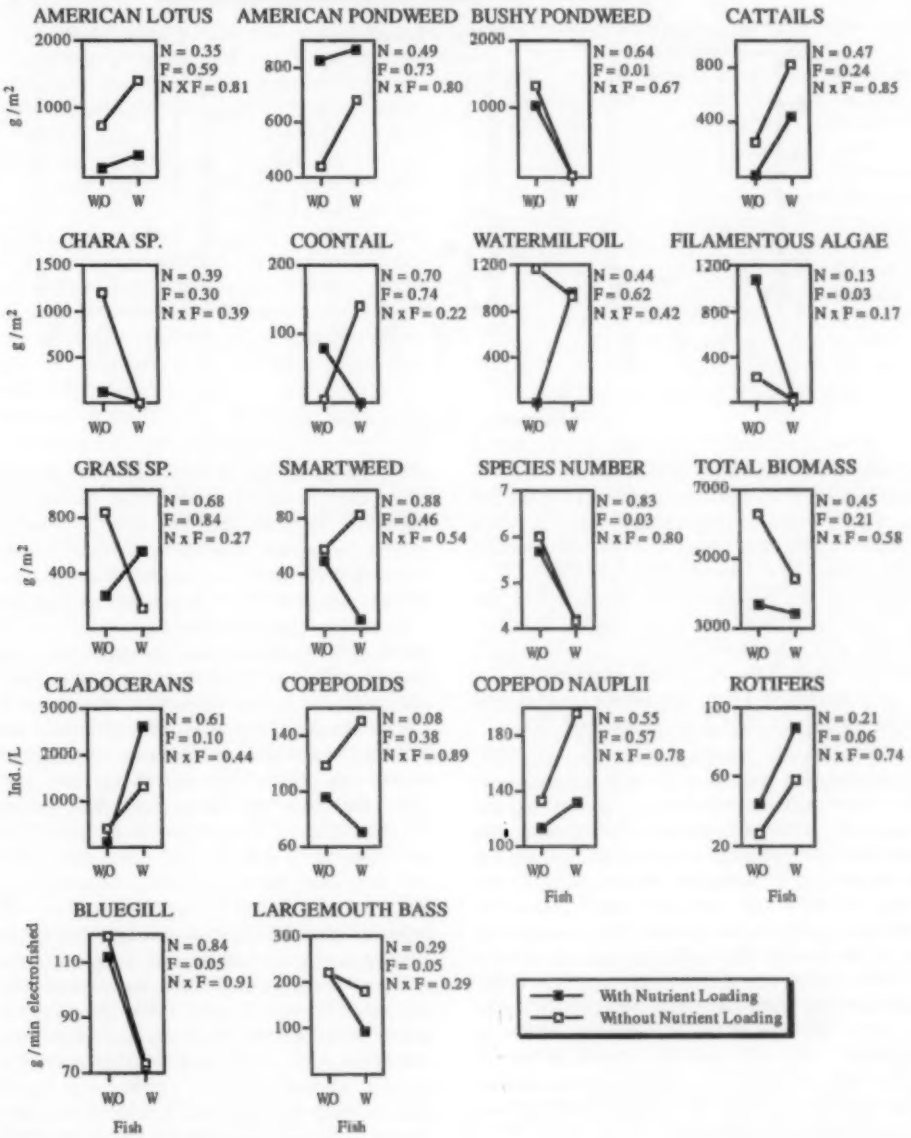
Fig. 5. Mean responses of macrophytes, zooplankton, and fish to nutrients and omnivorous fish (carp) manipulations in the pond experiment. Each point represents the mean of the replicate ponds within the treatment combination. Probability values from ANOVA of effects of nutrient loading effects (N), omnivorous fish effects (F), and their interaction effects (N × F) are indicated to the right of each graph. W, With omnivorous fish; W/O, without omnivorous fish.



trofishing were also lower in the ponds with carp (Fig. 5), but the lower catch rates for centrarchids could be due to declines in the efficiency of electrofishing with turbidity and not actual decreases in the populations of centrarchids.

Discussion

The primary objective of the mesocosm and pond experiments presented here were to test Drenner et al.'s (1996) hypothesis that nutrient loading and omnivorous fish operate synergistically to enhance phytoplankton biomass. In these experiments we evaluated the interdependence of nutrient loading and omnivorous fish effects as nutrient–fish interaction effects on chlorophyll. In the mesocosm experiment we observed a nutrient–fish synergism for primary productivity and chlorophyll in the presence of tilapia and carp, but in the pond experiment with carp we found little evidence that chlorophyll was regulated by a nutrient–fish synergism. We did not detect a nutrient–fish interaction effect for chlorophyll during the first fertilization period (March–May 1992) of the pond experiment. The absence of a nutrient–fish synergism for chlorophyll during the first fertilization period of

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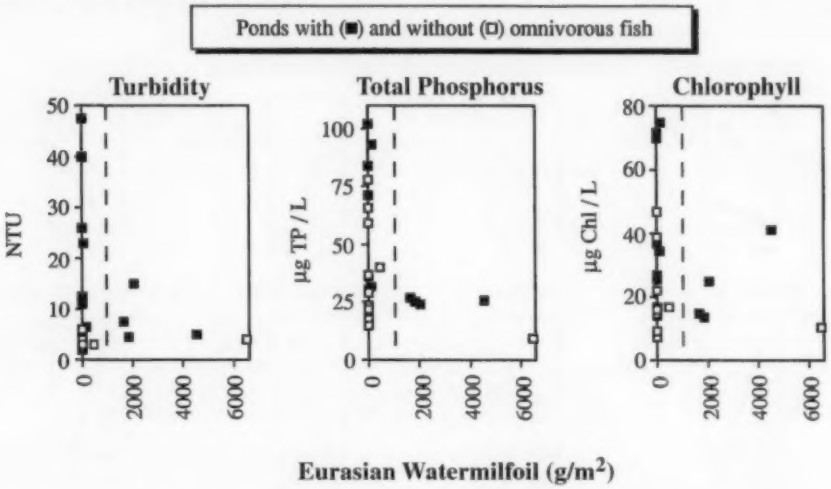
Fig. 6. Turbidity, TP, and chlorophyll plotted as functions of Eurasian watermilfoil biomass in ponds during August 1993. Broken line is the hypothesized threshhold (1 kg/m^2) for watermilfoil effects.



the experiment may have been due, in part, to the low biomass of carp and low levels of nutrient loading at the start of the experiment. The biomass of carp stocked into the ponds was approximately 7 kg/ha, which was less than the biomass of carp (22 kg/ha) found in reservoirs (Miranda 1983). Also nutrient loading during the first spring and summer was low (0.29 kg P·ha^{-1}·day^{-1} and 0.09 kg N·ha^{-1}·day^{-1}), approximately 16 and 14% of the rates of phosphorus and nitrogen loading, respectively, used in the mesocosm experiment. Nutrient loading only increased TP in the ponds in June and July (1992) while having no significant effects on TN.

In the second fertilization period (March–August 1993) we found a trend toward a nutrient–fish synergism in June and July with chlorophyll concentrations greater in the ponds with carp and nutrients compared with ponds with carp and no nutrients, but the nutrient–fish synergerism was not statistically significant until August. By the second summer, the biomass of carp had increased since fish were stocked, and nutrient loading had a greater effect on nutrient concentrations in the ponds. Electrofishing surveys of the ponds in fall 1993 showed that the average weight of individual carp had increased from a stocked weight of 5.7 g to a weight of 1.2 kg at the end of the experiment. Although nutrient loading rates were still low (0.21 kg P·ha^{-1}·day^{-1} and 0.06 kg N·ha^{-1}·day^{-1}) during the spring and summer of 1993, cumulative nutrient loading was sufficient to increase TP throughout the spring and summer.

We hypothesize that several mechanisms may be responsible for the nutrient–fish synergism for phytoplankton biomass in the ponds in August. When nutrients are added to lakes without omnivorous fish, the nutrients are transferred from the water column to the nutrient sink associated with bottom sediments via sedimentation of plankton and detritus. If bottom-feeding omnivorous fish are present, they act as nutrient pumps and transfer nutrients from the sediments to the water column where the nutrients stimulate primary productivity and chlorophyll biomass (Lamarra 1975; Brabrand et al. 1990; Mather et al. 1995; Schaus et al. 1997). The potential for omnivorous fish to affect the water column by nutrient pumping would intensify with nutrient loading, because there would be more food for the fish to consume. Moreover increased nutrient loading would also result in a greater biomasses of omnivorous fish. Lazzaro et al. (1992) found that the impact of omnivorous fish on chlorophyll and primary productivity is dependent on fish biomass.

In theory, clay resuspension by feeding activities of omnivorous fish could affect the interrelationship between nutrient loading and fish because suspended clay can alter plankton community responses to nutrient loading (Cuker 1993). The net effect of nutrient pumping versus clay resuspension was that carp increased chlorophyll in the mesocosms and ponds even though the carp resuspended clay. Also, the three-way interaction effect (nutrients–fish–clay) for chlorophyll in the mescosom experiment was not significant, indicating that the interaction effect between nutrients and fish were not significantly affected by clay. Thus we found no evidence that clay resuspension was an important factor affecting the interaction between nutrients and fish.

Suppression of herbivorous zooplankton by omnivorous fish was not responsible for the nutrient–fish synergism in the ponds during August 1993. Zooplankton samples collected in August revealed increased densities of cladoceran zooplankton (*Bosmina* and *Diaphanosoma*) in the presence of carp. Richardson et al. (1990) also found that the presence of carp was associated with increased populations of *Bosmina*. Several mechanisms may be responsible for increased cladoceran populations in the ponds with carp. Zooplankton birth rates may have been increased by enhancement of phytoplankton biomass in the presence of carp. Zooplankton death rates may have been decreased by the increase in turbidity and the resulting inability of zooplanktivorous fish to locate and feed upon macrozooplankton (Vinyard and O'Brien 1976). In addition, the biomass of bluegill and largemouth bass may have been lower in the presence of carp.

Although many limnologists recognize the power of experiments to test hypotheses about lake systems, controversy has existed over the relative merits of small-scale mesocosm

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experiments versus larger scale pond or lake experiments (Carpenter and Kitchell 1988; Carpenter et al. 1995; Drenner and Mazumder 1998). The mesocosm and pond experiments presented here illustrate the complementary relationship between small- and large-scale experiments. For example, in an earlier mesocosm experiment (Drenner et al. 1996) and the mesocosm experiment presented here, we found a novel interrelationship between omnivorous fish and nutrient loading in which the combination of omnivorous fish and nutrient loading had a greater effect on chlorophyll than the sum of their separate effects. However, in the pond experiment, we found little evidence that the nutrient–fish synergism regulated chlorophyll. In part, the absence of nutrient–fish synergism during the first year of the pond experiment was due to the low biomass of carp stocked and cold winter temperatures, which reduce fish activity. In addition, some ponds developed dense populations of Eurasian watermilfoil, which reduced concentrations of total phosphorus and chlorophyll, especially in the carp ponds. We hypothesize that Eurasian watermilfoil played a role in preventing the nutrient–fish synergism from affecting chlorophyll. We conclude that short-term mesocosm experiments are time- and cost-efficient tools to examine unexplored ecological interrelationships, but their results should be treated as hypotheses that should be further tested in larger and more environmentally complex systems.

Acknowledgments

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Early survival and development of coastal cutthroat trout (*Oncorhynchus clarki clarki*), steelhead (*Oncorhynchus mykiss*), and reciprocal hybrids

Denise K. Hawkins and Chris J. Foote

Abstract: We compared the survival and development of embryos from fertilization to emergence of two populations of coastal cutthroat trout (*Oncorhynchus clarki clarki*), one population of steelhead (*Oncorhymchus mykiss*), and their reciprocal hybrids under controlled conditions (10 ± 1°C). The egg weight of the steelhead (0.110 ± 0.008 (SE) g) was nearly twice that of coastal cutthroat trout (0.056 ± 0.003 and 0.051 ± 0.001 g for the two populations). Fertilization success, survival, duration of hatching and emergence periods, yolk absorption relative to total alevin weight, and yolk conversion efficiency were similar among the cross types. There was no evidence of reduced hatchability or viability of hybrids despite maternal and paternal species effects on size and development. Fish with cutthroat dams had lower percent hatch, were smaller at hatch and emergence, grew more slowly, used less yolk per day, reached 50% yolk absorption earlier, and had a smaller percent yolk at hatch than those fish with steelhead dams. Fish sired by cutthroat males hatched and emerged earlier and took less time between the two stages than fish sired by steelhead. As a consequence, hybrids with cutthroat dams emerged late and had little yolk, while hybrids with steelhead dams emerged early and had an abundance of yolk.

Résumé : Nous avons comparé les taux de survie et de développement d'embryons, de la fécondation à l'émergence, de deux populations côtières de truite fardée (*Oncorhynchus clarki clarki*), d'une population de truite arc-en-ciel anadrome (*O. mykiss*) et de leurs hybrides réciproques dans des conditions particulières (10 ± 1°C). Le poids des oeufs de la truite arc-en-ciel (0,110 ± 0,008 (é-t) g) était presque le double de celui de la truite fardée (0,056 ± 0,003 g et 0,051 ± 0,001 g pour les deux populations). La réussite de la fécondation, la survie, la durée des périodes d'éclosion et d'émergence, l'absorption du vitellus par rapport au poids total de l'alevin et l'efficacité de conversion du vitellus étaient semblables chez les types de croisements. Rien n'indiquait une baisse de la capacité d'éclosion ou de la viabilité des hybrides en dépit des effets des espèces maternelles et paternelles sur la taille et le développement. Les poissons ayant pour mère une truite fardée présentaient un pourcentage d'éclosion inférieur, étaient plus petits au moment de l'éclosion et de l'émergence, avaient une croissance plus lente, utilisaient moins de vitellus par jour, atteignaient le taux d'absorption de 50% du vitellus plus rapidement et disposaient d'un plus petit pourcentage de vitellus à l'éclosion comparativement aux poissons dont la mère était une truite arc-en-ciel. Les poissons dont le père était une truite fardée présentaient une éclosion et une émergence plus hâtives et la période entre ces deux étapes était plus courte que chez les poissons dont le père était une truite arc-en-ciel. Les hybrides ayant pour mère une truite fardée émergeaient tardivement et disposaient de peu de vitellus tandis que les hybrides ayant une truite arc-en-ciel anadrome pour mère émergeaient plus tôt et disposaient de beaucoup de vitellus.

[Traduit par la Rédaction]

Introduction

Hybridization can be defined as the breakdown of isolating mechanisms between two reproductively isolated species or populations. In fishes, hybridization is common within many families, due in large part to external fertilization and weak pair bonds between the parents (Avise 1994). There are many examples of hybridization among salmonids (reviewed by Verspoor and Hammar 1991). In a review of the fitness of hybrids relative to their parents, Arnold and Hodges (1995) provided examples from many taxa and genera and concluded that hybrids are not uniformly less fit than the parental species. Although the relative fitnesses of hybrid and pure-strain offspring have seldom been compared in salmonids, superior performance by hybrids is generally the exception (Leary et al. 1995). The relative fitness of F_1 hybrids determines whether occasional hybridization strengthens isolating mechanisms or leads to introgression of the gene pools (Avise 1994).

Behnke (1988) divided cutthroat trout (*Oncorhynchus clarki*) into four major subspecies and 10 minor subspecies depending on historical biogeography and the magnitude of phylogenetic divergence. Introgression between many of the interior subspecies of cutthroat trout and introduced rainbow

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Fig. 1. Genetic design of cutthroat trout and steelhead crosses. The female parent is listed first in all cross types (C, cutthroat; S, steelhead). Subscripts indicate hatchery of origin for that parent (a, Aberdeen; s, Shelton). This design was repeated five times. Cutthroat stocks were subsequently pooled for analyses when no differences were found between them.

	C_aC_a Aberdeen cutthroat dam	C_sC_s Shelton cutthroat dam	SS steelhead dam
C_aC_a Aberdeen cutthroat sire	C_aC_a Aberdeen pure cutthroat	Cross not done	S_aC_a hybrid steelhead Aberdeen sire
C_sC_s Shelton cutthroat sire	Cross not done	C_sC_s Shelton pure cutthroat	S_sC_s hybrid steelhead Shelton sire
SS steelhead sire	C_aS_a hybrid cutthroat Aberdeen dam	C_sS_s hybrid cutthroat Shelton dam	SS pure steelhead

trout (*Oncorhynchus mykiss*) is widespread (Forbes and Allendorf 1991). This propensity to complete introgression suggests that because the interior subspecies of cutthroat trout were isolated from rainbow trout, they did not evolve isolating mechanisms to prevent hybridization (Behnke 1992).

Coastal cutthroat trout (*Oncorhynchus clarki clarki*) coexist with steelhead (the anadromous form of *O. mykiss*) throughout much of their native range. Although the timing of their phyletic divergence is not fully known (Utter and Allendorf 1994), Behnke (1992) concluded that cutthroat trout and steelhead shared a common ancestor as recently as 2 million years ago. As would be expected from this relatively recent divergence, cutthroat trout and steelhead share many characteristics. However, differences at protein-coding loci (e.g., Leary et al. 1987), in mtDNA sequence (Wilson et al. 1985), in karyotype (Gold 1977), and in morphology (e.g., Hawkins and Quinn 1996) can be used to distinguish between the species.

There are several differences between coastal cutthroat trout and steelhead that could lead to both pre- and postmating reproductive isolating mechanisms that maintain the species' integrity. Spawning adults are generally separated both temporally and spatially (Trotter 1987; Burgner et al. 1992). In western Washington State, steelhead often spawn later in the season, using the deeper, faster water of larger rivers, whereas coastal cutthroat trout spawn earlier in smaller headwaters (Johnston and Mercer 1976). Steelhead are also often much larger at maturity, which could lead to assortative mating or possibly to directionality in pair formation in the event of heterospecific matings (Foote and Larkin 1988). Coastal cutthroat trout and steelhead also have different numbers of chromosomes (68 and 58–60, respectively; Behnke 1970), which could lead to mispairing of chromosomes and decreased viability in hybrid progeny. Furthermore, juvenile coastal cutthroat trout and steelhead use different stream habitats when sympatric and the hybrid progeny that do survive may be at a competitive disadvantage (Hawkins 1997).

Prior to 1981, hybridization between coastal cutthroat trout and steelhead was thought to be uncommon (Utter 1981), presumably due to the coevolution of coastal cutthroat trout and steelhead (Behnke 1992). However, Campton and Utter (1985) found a substantial number of hybrids in two streams in the Puget Sound area of Washington. Hybridization is cause for concern because it may contribute to the decline of western trout species (Busack and Gall 1981), and many cutthroat trout populations currently are declining (Johnson et al. 1994). Allendorf and Leary (1988) concluded that hybridization has been the main impact of introduced fish on native cutthroat trout populations. The incidence of natural hybrids between coastal cutthroat trout and steelhead, coupled with the apparent ability of the two species to maintain their genetic integrities in areas of natural sympatry, raises many questions regarding the existence and effectiveness of potential postzygotic isolating mechanisms.

Here, we examine (*i*) the relative viabilities of coastal cutthroat trout (subsequently referred to simply as cutthroat), steelhead, and their reciprocal hybrids when raised under controlled conditions from fertilization to 64 days postfertilization and (*ii*) the development of the embryos to distinct stages from fertilization to the voluntary emergence of the alevins. We report no evidence of decreased viability of hybrids, but did detect genetic differences in development rate.

Methods

Study populations

Adults from two populations of cutthroat trout (Lake Aberdeen Hatchery, Aberdeen, Wash., and Eels Springs Hatchery, Shelton, Wash.) and one population of steelhead (Lake Aberdeen Hatchery) were used to create the progeny for this experiment. Both populations of cutthroat originated from anadromous fish, but are currently maintained as captive broodstocks whereas the steelhead are released into the wild as smolts. The Lake Aberdeen cutthroat trout is the product of hybridization with steelhead in the past. Protein electrophoresis suggests that the majority of the adults used had cutthroat alleles at all diagnostic loci (Hawkins 1997).

Fertilization and rearing

Eggs and milt were obtained on 25 January 1995 from the two hatcheries and transported separately in plastic bags on ice to the University of Washington, School of Fisheries hatchery. Two hours later, the eggs from each cutthroat or steelhead female were divided and fertilized by milt from both cutthroat and steelhead males as detailed in Fig. 1. Five males and five females from each population were used, resulting in 35 families. The four major cross types were as follows: CC, pure cutthroat; CS, hybrids with cutthroat dams (referred to as hybrid cutthroat); SC, hybrids with steelhead dams (referred to as hybrid steelhead); SS; pure steelhead. Because two cutthroat populations were used, the crosses involving cutthroat can be subdivided into six minor cross types: C_aC_a, pure Aberdeen cutthroat; C_sC_s, pure Shelton cutthroat; C_aS_a, hybrid cutthroat with Aberdeen cutthroat dams; C_sS_s, hybrid cutthroat with Shelton cutthroat dams; S_aC_a, hybrid steelhead with Aberdeen cutthroat sires; S_sC_s, hybrid steelhead with Shelton cutthroat sires. This experimental design resulted in five independent replicates of a group of seven connected families. Within a group, the pure families (CC, SS) were not related; likewise, the hybrid cutthroat and hybrid steelhead (CS, SC) were not related. However, the hybrid types were related to two of the pure families and to like hybrids. Families within a row were paternal half-sibs and those within a column were maternal half-sibs (Fig. 1). Among the five replicates, none of the families were related.

Each family was incubated in a randomly assigned Heath tray in dechlorinated city water at 10 ± 1°C. At 21 days postfertilization, at the eyed-embryo stage of development, eggs and embryos from

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all families were mechanically shocked by pouring them onto a damp tray from a height of 10 cm to facilitate identification and removal of all unfertilized eggs and dead embryos. All remaining embryos were counted for each family. All of the embryos from two cutthroat females from Aberdeen, accounting for two pure cutthroat and two hybrid cutthroat families, did not develop due to poor egg quality. One hundred embryos per family were then placed in two egg cups located in each Heath tray (50 per cup) to control for density and the amount of swimming space available to the alevins after hatching. Embryos were divided equally into the two cups for families with fewer than 100 embryos, and all embryos were placed in one cup for families with fewer than 50 embryos. These embryos were used to determine the rate of hatching, growth, and yolk absorption and the duration of the hatching period. The remaining embryos were left in the Heath tray for estimating survival. The cups were made from 10-cm-diameter PVC pipe with window screen on one side. The top screen of the Heath tray prevented embryos from entering or leaving the cups after hatching.

At the completion of hatching, 50 embryos (those in one egg cup) from each of three families per minor cross type and pure steelhead were placed into emergence chambers to determine the length of time from fertilization to 50% emergence, duration of emergence period (time from emergence of first to last fish), and length of time between 50% hatch and 50% emergence. The emergence chambers allowed the embryos to hide in the substrate and volitionally emerge (Hendry 1995). The remaining embryos were left in their egg cups (used for determination of growth and yolk absorption) or loose in the Heath trays. At this point, all embryos and alevins were switched from dechlorinated city water to Lake Washington water at 10 ± 1°C. As the alevins in the Heath trays reached the swim-up stage of development (indicated by behavior), they were placed in 10-L buckets for continued rearing and survival measurements by family.

Data collection

Egg size was determined from 10 eggs per female collected prior to fertilization and preserved in 10% buffered formalin. After 2 months in formalin, each egg was blotted on paper towel to remove excess formalin and weighed to ± 0.001 g with a digital Mettler balance.

To minimize fungal growth, eggs or alevins that were not developing were removed and counted 1 day after fertilization, at the eyed-embryo stage, periodically until hatching, once every day during hatching, and every fourth day thereafter. All removed eggs were cleared with Stockard's solution to determine unfertilized eggs and stage of death of fertilized eggs. There was no particular stage at which development stopped within any of the cross types, and the results are not presented here. Survival was compared for all families at three intervals of development: fertilization to eyed embryo, eyed embryo to hatch, and hatch to 64 days of age. Percent mortality of each family was calculated as the number of dead eggs divided by the total starting number of eggs at each of the three intervals. Fertilization success was determined as the percentage of eggs that were fertilized after blank and atritic eggs (eggs that did not contain a yolk or a yolk that was partially resorbed) were removed from the total. Hatchability of each family was calculated as the percentage of fertilized eggs that hatched.

Throughout the hatching period, all hatched embryos were counted in each egg cup three times per day. Length of time from fertilization to 50% hatch and duration of hatching period (time from the first to the last embryo hatched) were determined for each family from the embryos in the egg cups. When the last embryo had hatched within a family, three hatched embryos were randomly selected to determine the body weight and amount of yolk at hatching. After preservation for at least 1 month, the yolk was dissected from the body and both were dried for 24 h at 95°C to remove all moisture and then weighed to ± 0.001 g.

For the collection of the emergence data, the hatched embryos were added to the emergence chamber and within 15 min had all moved down into the marbles. The emergence chambers consisted of an inner chamber of water about 30% full of marbles with an exit notch at the top, within an outer chamber of water. As alevins emerged, they moved from the inner chamber to the outer chamber through the exit notch at the top of the inner chamber. Once in the outer chamber, they were unable to reenter the inner chamber because of the water level difference between the two (10 cm). Throughout the emergence period, alevins in the outer chamber were counted twice daily and removed. The fifth, 20th, and 45th alevins to emerge from each family were collected for determination of body weight and amount of yolk at emergence.

The absolute rate of yolk absorption, yolk absorption relative to total embryo weight, and rate of growth were determined from samples of the 50 embryos left in the remaining egg cups in the Heath trays. Beginning at 40 days postfertilization, three embryos from each family were sampled for body weight and amount of yolk every fourth day. Samples were taken until 64 days postfertilzation, at which time, all fish in families had completely absorbed their yolk sacs. Length of time postfertilization to 50% yolk absorption and yolk conversion efficiency were also determined from these samples. Yolk conversion efficiency from day 40 to day 52 postfertilization (change in body weight/change in yolk weight) was calculated for all families with embryos in egg cups (except one hybrid steelhead family whose samples were lost during processing).

Statistical analyses

All measurements of percent fertilization, hatched, dead, and yolk were transformed using an arcsine square root transformation to normalize the data before either ANOVA or regression analysis. Because there were no differences between the two populations of cutthroat in either the pure cutthroat families or the reciprocal hybrid families (ANOVA), the Shelton and Aberdeen cutthroat populations were pooled for the analyses presented here.

Length of time to 50% hatch, 50% emergence, and 50% yolk were interpolated for each family using the regression equation from the linear portion of the graph of cumulative percent hatched, or emerged or percent yolk against number of days postfertilization.

A two-way ANOVA model was used to examine the effects of dam type, sire type, and interaction between dam and sire types among the four major cross types (CC, CS, SC, SS) (family means were used in all tests to avoid pseudoreplication):

$$Y_{ijk} = \mu_{..} + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \epsilon_{ijk}$$

where $\mu_{..}$ is a constant, α_i is the effect of the dam type, β_j is the effect of the sire type, $(\alpha\beta)_{ij}$ is the interaction effect between the dam and sire types, and ϵ_{ijk} is the independent error term.

When significant dam type effects were identified for a trait, simple linear regression of that trait on egg weight was carried out within each cross type to determine whether the results were due to species differences or could be explained by differences in egg size alone. ANCOVA was not appropriate because the egg sizes of cutthroat and steelhead did not overlap.

To examine the phenotypic correlations among the many traits measured in this study, Spearman correlation coefficients were calculated using the data from the 21 families for which all the traits were measured. The traits with significant dam type effects were generally correlated whereas the traits with significant sire type effects were correlated.

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Table 1. Group means and results of statistical analyses for two-way ANOVA tests of dam type, sire type, and interaction effects.

Trait	CC mean (SE)	CS mean (SE)	SC mean (SE)	SS mean (SE)	Dam type effect	Sire type effect	Interaction effect dam type × sire type
Fertilization (%)	85.304 (7.074)	85.301 (6.038)	98.022 (0.896)	95.482 (3.155)	$F_{1,27} = 8.749$ $p = 0.006$	$F_{1,27} = 0.232$ $p = 0.634$	$F_{1,27} = 0.213$ $p = 0.648$
Fertilization to eyed-embryo mortality (%)	3.266 (1.421)	3.858 (2.683)	0.078 (0.053)	0.278 (0.278)	$F_{1,27} = 13.605$ $p = 0.001$	$F_{1,27} = 0.031$ $p = 0.862$	$F_{1,27} = 0.236$ $p = 0.631$
Eyed-embryo to hatch mortality (%)	0.885 (0.413)	0.866 (0.460)	0.181 (0.100)	0.100 (0.100)	$F_{1,27} = 4.871$ $p = 0.036$	$F_{1,27} = 0.028$ $p = 0.860$	$F_{1,27} = 0.060$ $p = 0.808$
Hatch to 64 days mortality (%)	1.918 (0.463)	1.755 (0.673)	1.295 (0.297)	0.716 (0.244)	$F_{1,27} = 2.334$ $p = 0.138$	$F_{1,27} = 0.799$ $p = 0.379$	$F_{1,27} = 0.238$ $p = 0.629$
Hatchability (%)	95.432 (1.506)	95.199 (2.908)	99.689 (0.131)	99.688 (0.138)	$F_{1,27} = 16.070$ $p < 0.001$	$F_{1,27} = 0.040$ $p = 0.843$	$F_{1,27} = 0.110$ $p = 0.743$
Duration of hatching (days)	1.381 (0.135)	1.469 (0.189)	1.295 (0.161)	1.970 (0.353)	$F_{1,27} = 1.078$ $p = 0.308$	$F_{1,27} = 3.638$ $p = 0.067$	$F_{1,27} = 2.160$ $p = 0.153$
Time to 50% hatch (days)	32.193 (0.175)	32.848 (0.219)	32.784 (0.113)	33.890 (0.280)	$F_{1,27} = 18.410$ $p < 0.001$	$F_{1,27} = 21.421$ $p < 0.001$	$F_{1,27} = 1.406$ $p = 0.246$
Body weight at hatch (g)	0.004 (0.0002)	0.0042 (0.0002)	0.0065 (0.0002)	0.0067 (0.0002)	$F_{1,27} = 153.021$ $p < 0.001$	$F_{1,27} = 0.588$ $p = 0.450$	$F_{1,27} = 0.003$ $p = 0.954$
Yolk at hatch (%)	78.39 (0.70)	78.04 (0.76)	84.03 (0.75)	83.34 (1.46)	$F_{1,27} = 37.529$ $p < 0.001$	$F_{1,27} = 0.324$ $p = 0.574$	$F_{1,27} = 0.039$ $p = 0.844$
Duration of emergence (days)	11.65 (1.76)	10.78 (1.40)	14.21 (1.52)	19.41 (1.25)	$F_{1,17} = 11.074$ $p = 0.004$	$F_{1,17} = 1.658$ $p = 0.215$	$F_{1,17} = 3.245$ $p = 0.089$
Time to 50% emergence (days)	56.40 (0.45)	58.57 (0.44)	55.55 (0.23)	58.81 (1.45)	$F_{1,17} = 0.285$ $p = 0.600$	$F_{1,17} = 23.045$ $p < 0.001$	$F_{1,17} = 0.912$ $p = 0.353$
Body weight at emergence (g)	0.013 (0.0007)	0.014 (0.0008)	0.024 (0.0014)	0.025 (0.0023)	$F_{1,17} = 78.117$ $p < 0.001$	$F_{1,17} = 0.627$ $p = 0.439$	$F_{1,17} = 0.061$ $p = 0.809$
Yolk at emergence (%)	16.46 (3.52)	9.81 (1.19)	39.33 (2.23)	35.31 (6.21)	$F_{1,17} = 60.490$ $p < 0.001$	$F_{1,17} = 3.247$ $p = 0.089$	$F_{1,17} = 0.425$ $p = 0.523$
Time between 50% hatch and 50% emergence (days)	24.16 (0.38)	25.85 (0.38)	22.63 (0.20)	24.90 (1.66)	$F_{1,17} = 4.729$ $p = 0.044$	$F_{1,17} = 12.048$ $p = 0.003$	$F_{1,17} = 0.263$ $p = 0.614$
Growth: 40–52 days postfertilization (g)	0.0068 (0.0003)	0.0067 (0.0004)	0.0130 (0.0009)	0.0135 (0.0010)	$F_{1,26} = 81.109$ $p < 0.001$	$F_{1,26} = 0.089$ $p = 0.768$	$F_{1,26} = 0.206$ $p = 0.654$
Yolk conversion efficiency: 40–52 days postfertilization	0.711 (0.033)	0.788 (0.052)	0.688 (0.019)	0.830 (0.039)	$F_{1,26} = 0.063$ $p = 0.804$	$F_{1,26} = 8.230$ $p = 0.008$	$F_{1,26} = 0.734$ $p = 0.399$
Time to 50% yolk (days)	42.84 (0.33)	43.68 (0.66)	47.53 (0.46)	48.49 (0.71)	$F_{1,27} = 73.763$ $p < 0.001$	$F_{1,27} = 2.680$ $p = 0.113$	$F_{1,27} = 0.010$ $p = 0.921$
Change in yolk weight: 40–52 days postfertilization (g)	0.0097 (0.0006)	0.0087 (0.0008)	0.0187 (0.0008)	0.0166 (0.0018)	$F_{1,26} = 77.741$ $p < 0.001$	$F_{1,26} = 2.663$ $p = 0.115$	$F_{1,26} = 0.379$ $p = 0.543$

Note: In all groups, the species of the dam is listed first (C, cutthroat; S, steelhead). The group means are untransformed means in the case of percentage data. All percentage data were transformed prior to ANOVA.

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Results

The group means and ANOVA results are given in Table 1 for all of the traits except egg weights, which are given below.

Egg weight

The average egg weight of the two stocks of cutthroat was similar (Tukey $P > 0.8$; Aberdeen: 0.056 ± 0.003 SE g, range 0.053–0.063 g; Shelton: 0.051 ± 0.001 g, range 0.048–0.055 g) and less than that for steelhead (0.110 ± 0.008 g, range 0.091–0.134 g; $F_{2,10} = 38.270$, $P < 0.001$; Tukey $P < 0.001$ for steelhead compared with both Aberdeen and Shelton cutthroat).

Traits with significant dam type effects

Dam type effects were significant for the following traits: fertilization, mortality from fertilization to the eyed-embryo stage and from the eyed-embryo stage to hatching, hatchability, body weight and percent yolk at both hatching and emergence, duration of the emergence period, growth from 40 to 52 days postfertilization (Fig. 2A), length of time to 50% yolk, and yolk absorption from 40 to 52 days postfertilization (Fig. 2B) (Table 1).

Steelhead eggs were nearly twice the weight of cutthroat eggs. Consequently, maternal steelhead crosses were larger with a higher percentage of yolk at hatch, grew faster, and were larger with a higher percentage of yolk at emergence than maternal cutthroat (both pure and hybrid) (Figs. 2A and 2B). Because yolk absorption was proportional to body weight across species (larger fish use the same amount of yolk relative to their body weight, but use absolutely more yolk than smaller fish), the decrease in absolute yolk weight was higher in maternal steelhead than in maternal cutthroat. The combination of proportional absorption of yolk and a higher percent yolk at hatch in maternal steelhead resulted in a longer time from fertilization until 50% yolk absorption in maternal steelhead compared with maternal cutthroat (Fig. 2B).

Traits with significant sire type effects

Sire type effects were significant for the time to 50% emergence and yolk conversion efficiency from 40 to 52 days postfertilization (Table 1).

Traits with significant dam and sire type effects

The following traits had both significant dam type and sire type effects (the interaction effects between the dam and sire types were not significant): length of time to 50% hatch and length of time between hatching and emergence (Table 1). These sex-specific effects acted together on hatching time. Steelhead sires and dams increased the time to hatching. Therefore, steelhead hatched later than all other cross types, cutthroat hatched earlier, and hybrids were generally intermediate (Fig. 2C). For the time between hatching and emergence the sex-specific effects acted in opposition within a species: steelhead sires increased the time and steelhead dams decreased the time. However, the sire type effect dominated so that pure steelhead took longer to emerge after hatching than pure cutthroat. Because of the opposing sex-specific effects on this trait, the means of the hybrids were extreme compared with the means of the pure species. Hybrid steelhead had the shortest time between hatching and emergence, due to the influence of steelhead dams and cutthroat sires, and hybrid cutthroat had the opposite influence from cutthroat dams and steelhead sires and took longer between hatching and emergence than all other cross types. There was also a strong sire type effect on time to emergence that enhanced the difference in time between hatching and emergence. Steelhead sires increased and cutthroat sires

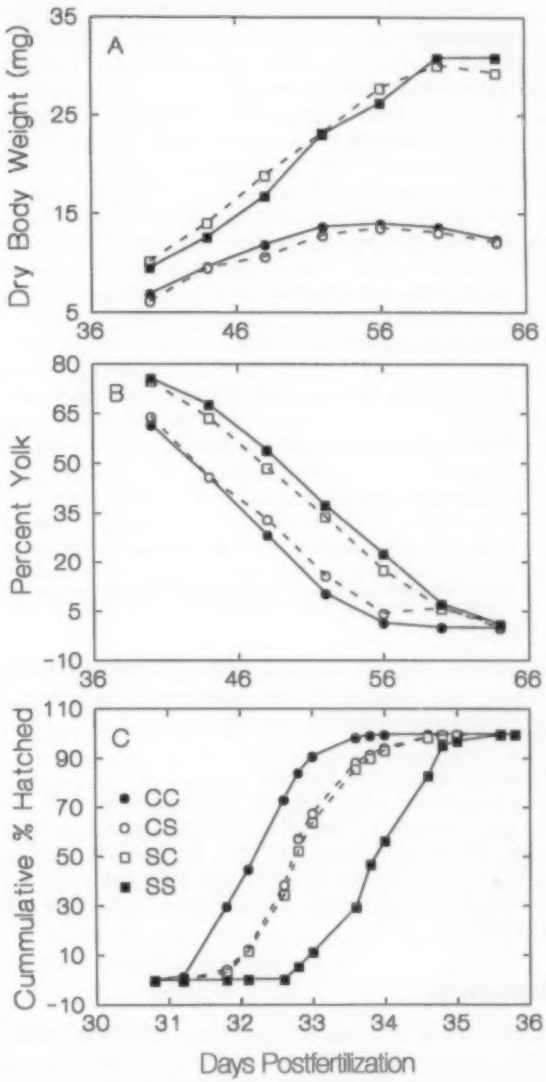

Fig. 2. (A) Plot of body weight by time for pure and reciprocal hybrids of steelhead, cutthroat trout, and reciprocal hybrids. (B) Rate of yolk absorption relative to total embryo weight for each cross type. For analyses presented in the text, slopes and change in body weight were calculated over the linear portion of the graph (40–52 days postfertilization). (C) Mean cumulative percent hatched for each cross type. The species of the dam is listed first in all cross types (C, cutthroat; S, steelhead).

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Table 2. Simple linear regression analyses of each trait on egg weight for those traits with significant dam type effects.

Trait	CC	CS	SC	SS
Fertilization (%)	$r^2 = 0.009$	$r^2 = 0.020$	$r^2 = 0.235$	$r^2 = 0.100$
	$p = 0.824$	$p = 0.735$	$p = 0.156$	$p = 0.604$
Fertilization to eyed-embyo mortality (%)	$r^2 = 0.010$	$r^2 = 0.011$	$r^2 = 0.097$	$r^2 = 0.124$
	$p = 0.814$	$p = 0.804$	$p = 0.381$	$p = 0.561$
Eyed-embro to hatch mortality (%)	$r^2 = 0.005$	$r^2 = 0.095$	$r^2 = 0.001$	$r^2 = 0.561$
	$p = 0.866$	$p = 0.457$	$p = 0.932$	$p = 0.145$
Hatchability (%)	$r^2 = 0.002$	$r^2 = 0.007$	$r^2 = 0.009$	$r^2 = 0.021$
	$p = 0.921$	$p = 0.842$	$p = 0.789$	$p = 0.815$
Time to 50% hatch (days)	$r^2 = 0.355$	$r^2 = 0.000$	$r^2 = 0.013$	$r^2 = 0.129$
	$p = 0.119$	$p = 0.985$	$p = 0.749$	$p = 0.552$
Body weight at hatch (g)	$r^2 = 0.783$	$r^2 = 0.403$	$r^2 = 0.257$	$r^2 = 0.105$
	$p = 0.004$	$p = 0.091$	$p = 0.135$	$p = 0.595$
Yolk at hatch (%)	$r^2 = 0.009$	$r^2 = 0.588$	$r^2 = 0.737$	$r^2 = 0.921$
	$p = 0.823$	$p = 0.026$	$p = 0.001$	$p = 0.010$
Duration of emergence (days)	$r^2 = 0.005$	$r^2 = 0.040$	$r^2 = 0.169$	$r^2 = 0.001$
	$p = 0.894$	$p = 0.703$	$p = 0.417$	$p = 0.980$
Body weight at emergence (g)	$r^2 = 0.378$	$r^2 = 0.539$	$r^2 = 0.738$	$r^2 = 0.777$
	$p = 0.194$	$p = 0.097$	$p = 0.028$	$p = 0.313$
Yolk at emergence (%)	$r^2 = 0.006$	$r^2 = 0.077$	$r^2 = 0.469$	$r^2 = 0.248$
	$p = 0.886$	$p = 0.594$	$p = 0.133$	$p = 0.668$
Time between 50% hatch and 50% emergence (days)	$r^2 = 0.057$	$r^2 = 0.037$	$r^2 = 0.137$	$r^2 = 0.254$
	$p = 0.650$	$p = 0.717$	$p = 0.470$	$p = 0.664$
Growth: 40–52 days postfertilization (g)	$r^2 = 0.090$	$r^2 = 0.332$	$r^2 = 0.573$	$r^2 = 0.743$
	$p = 0.471$	$p = 0.135$	$p = 0.018$	$p = 0.060$
Time to 50% yolk (days)	$r^2 = 0.233$	$r^2 = 0.016$	$r^2 = 0.577$	$r^2 = 0.382$
	$p = 0.226$	$p = 0.762$	$p = 0.011$	$p = 0.267$
Change in yolk weight: 40–52 days postfertilization (g)	$r^2 = 0.152$	$r^2 = 0.460$	$r^2 = 0.643$	$r^2 = 0.745$
	$p = 0.340$	$p = 0.064$	$p = 0.009$	$p = 0.059$

Note: All percentage data were transformed prior to analysis. In all groups, the species of the dam is listed first (C, cutthroat; S, steelhead).

decreased the time to emergence. Hybrid steelhead emerged first, cutthroat emerged second, hybrid cutthroat emerged third, and steelhead emerged last (Table 1).

Egg weight regression analyses

Egg weight was correlated with body weight at hatch (for CC) and emergence (for SC), growth (change in body weight from 40 to 52 days) (for SC), percent yolk at hatch (for CS, SC, SS), length of time to 50% yolk absorption (for SC), and the change in yolk weight from 40 to 52 days (for SC) (Table 2).

Discussion

Hybridization often has adverse effects on fitness characteristics such as survival, growth, and development (Leary et al. 1995). Comparative studies of salmonid hybrid survival and early development often come to conflicting conclusions as to the relative performance of hybrid and pure species. Depending in large part on which traits are used to evaluate the relative performance, whether reciprocal hybrids are examined, and possibly which species are observed, the data show no difference among hybrids and pure species (e.g., Ferguson et al. 1988; Wood and Foote 1990), intermediate performance of hybrids (e.g., Dumas et al. 1992), superior performance by hybrids (e.g., Halliburton et al. 1983), and in some cases mixed results including all of the previous possibilities plus inferior performance by some hybrids (e.g., Halliburton et al. 1983; Ferguson et al. 1988; Wood and Foote 1990; Dumas et al. 1992; McGowan and Davidson 1992). In the study described here, the majority of the traits had a dam type effect with maternal cutthroat types similar to each other and different from maternal steelhead types. However, three measurements of development rate had a sire type effect, and thus, hybrids either grouped with the paternal species (two traits) or were intermediate (one trait) depending on the trait examined.

Relative survival as a component of fitness has been compared within species (e.g., Beacham 1988), between forms within species (e.g., Wood and Foote 1990, 1996), and between hybridizing species (e.g., Halliburton et al. 1983; Dumas et al. 1992; McGowan and Davidson 1992). Maternal effects accounted for most of the variation detected within species and between species; hybrids were not different from the maternally related pure species. Similarly, in this study, there was no evidence that fertilization success, hatchability, or subsequent survival of heterospecific crosses was less successful than for conspecifics in either steelhead or cutthroat trout. Therefore, decreased fertilization and viability of heterospecific embryos alone do not appear to function as postzygotic isolating mechanisms. For these three traits, there was a dam type effect with the hybrid crosses similar to the maternally related pure crosses. Maternal cutthroat demonstrated decreased fitness compared with mater-

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nal steelhead under the conditions of gamete collection and rearing of this study.

Many studies have shown that larger eggs produce larger embryos with greater yolk reserves at hatch and that these larger embryos grow faster and are larger with greater yolk reserves at emergence (e.g., Hayashizaki et al. 1995). In the study described here the results were consistent with the literature results: maternal steelhead were larger with more yolk and hybrids were not clearly at any disadvantage.

Length of time postfertilization to hatch and emergence and time between hatch and emergence are all measures of development influenced by the sire (Wood and Foote 1990; McGowan and Davidson 1992). Furthermore, development rate is matched to the amount of yolk reserves available for development, which is an egg size (maternal) effect (Wood and Foote 1990; McGowan and Davidson 1992). Consistent with these studies within species in *Oncorhynchus* and between species in *Salmo*, body weight, yolk reserves, and growth all had a strong dam type effect in the reported data. Furthermore, in this study between species in *Oncorhynchus*, the three measures of development rate (time to 50% hatch and emergence and length of time between 50% hatch and emergence) had a strong sire type effect. It is the combination of the dam type influence on size and yolk reserves and the sire type influence on development rate that leads to the potential for mismatch in development rate and egg size in hybrids (Wood and Foote 1990). In this study, hatching time (in agreement with the results of Dumas et al. (1992) on brook trout (*Salvelinus fontinalis*) – Arctic char (*Salvelinus alpinus*) hybrids) and time between hatching and emergence had both dam and sire type effects, and emergence timing had sire type effects that affected the relationship of the hybrids to the pure species. Although the hybrids were intermediate compared with the pure species at hatching time, they were extreme compared with the pure species in the time between hatching and emergence and thus emerged similarly to the paternally related pure species.

Survival and development of salmonid fry from fertilization to emergence appear to be under strong natural selection both among and within populations (Brannon 1987). For example, spawning date in sockeye salmon (*Oncorhynchus nerka*) was correlated with the temperatures experienced by developing young. Further, within spawning populations, those fish that spawned earlier in the run developed more slowly than those that spawned at the end of the run.

Other studies have concluded that emergence timing, size, and amount of yolk at emergence have fitness consequences (e.g., Brannas 1995). Early-emerging fish may be better able to obtain and defend a feeding territory if food is available, but may not be able to survive if the food source is cyclical (Bagenal 1969). However, alevins with large orange yolk sacs that could exist on yolk reserves while waiting for a cyclic food source are more vulnerable to predation because they are both more visible and less maneuverable (Fresh and Schroder 1987). Smaller fish also tend to be more vulnerable to predation (Parker 1971), and alevins from same-sized eggs but with larger yolk sacs at emergence tend to be smaller because they have not fully converted the yolk into body tissue (Wood and Foote 1990).

Within either cutthroat or steelhead, the rate of development is presumably matched to the body size and yolk reserves of the alevin so that the alevins emerge at an appropriate time and state of yolk absorption (Brannon 1987). However, cutthroat–steelhead hybrids are mismatched with either a fast rate of development in a large egg or a slow rate of development in a small egg so that they emerge with either too much or too little yolk relative to the pure species. Hybrid cutthroat had the small egg of a cutthroat and the slow development rate of a steelhead. They therefore emerged later than cutthroat and at a smaller size than steelhead, but with less yolk than either pure species. Hybrid steelhead had the large egg of a steelhead with the fast development rate of a cutthroat and thus face the opposite problem at emergence. They emerge earlier than the other cross types, and although they are larger than cutthroat, they have a very large remaining yolk sac. Therefore, depending on the conditions of food and predation present at emergence, the possibility exists that even if hybrids are fully viable developmentally, they may experience increased mortality relative to the pure species because of a mismatch between egg size and development rate.

This study provides further evidence of the divergence in development rates of closely related species. This divergence has both a maternal (egg size) and a genetic component. While we found no evidence of reduced viability of hybrids, the limited introgression of coastal cutthroat and steelhead in the face of interbreeding (Campton and Utter 1985; Hawkins 1997) indicates that selection against the hybrids most likely occurs. Given the known adaptive significance of development rates in salmonids (Brannon 1987), the mismatch in various aspects of development in coastal cutthroat and steelhead may play an important role in limiting introgression between the species.

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MULTIFAN-CL: a length-based, age-structured model for fisheries stock assessment, with application to South Pacific albacore, *Thunnus alalunga*

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Abstract: We introduce a length-based, age-structured model, MULTIFAN-CL, that provides an integrated method of estimating catch age composition, growth parameters, mortality rates, recruitment, and other parameters from time series of fishery catch, effort, and length frequency data. The method incorporates Bayesian parameter estimation, estimation of confidence intervals for model parameters, and procedures for hypothesis testing to assist model development. We apply the method to South Pacific albacore, *Thunnus alalunga*, fishery data and demonstrate the incorporation of model structure such as spatial heterogeneity, age-dependent natural mortality and movement rates, time series trends and seasonal variation in catchability, and density-dependent growth. Consistency of the results of the albacore analysis with various exogenous sets of biological and environmental data gives credence to the model results.

Résumé : Nous présentons un modèle fondé sur la longueur et structuré par l'âge, le MULTIFAN-CL, comme méthode intégrée pour l'estimation de la composition de l'âge des prises, des paramètres de croissance, du taux de mortalité, du recrutement, et d'autres paramètres, à partir de séries chronologiques de données sur les prises, l'effort et les fréquences de longueurs des captures. On y fait appel à l'estimation bayesienne de paramètres, à l'estimation des intervalles de confiance des paramètres du modèle ainsi qu'à des procédures de test d'hypothèses pour faciliter l'élaboration du modèle. Nous appliquons la méthode aux données sur la pêche du germon du Pacifique sud (*Thunnus alalunga*) et démontrons l'incorporation de structures comme l'hétérogénéité spatiale, la mortalité naturelle dépendante de l'âge et les taux de déplacement, les tendances des séries chronologiques et la variation saisonnière de la vulnérabilité à la pêche, et la croissance dépendante de la densité. La cohérence des résultats de cette analyse avec d'autres séries de données biologiques et environnementales rend crédibles les résultats obtenus avec le modèle.

[Traduit par la Rédaction]

Introduction

Age-structured models are now the method of choice for many fisheries stock assessments. Models range from simple deterministic methods, such as virtual population (or cohort) analysis (Megrey 1989), to statistical models in which variability in the data and various population processes is acknowledged (Doubleday 1976; Paloheimo 1980; Fournier and Archibald 1982; Pope and Shepherd 1982; Dupont 1983; Deriso et al. 1985; Schnute and Richards 1995; McAllister and Ianelli 1997).

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Statistical age-structured models are superior to deterministic models in that they permit the estimation of confidence intervals for the parameter estimates. This allows uncertainty in stock assessments to be incorporated into management advice through decision or risk analysis. Bayesian approaches to age-structured models (McAllister and Ianelli 1997; Punt and Hilborn 1997) now provide a powerful framework for undertaking integrated analysis of fish stocks and for expressing the full range of uncertainty in the resulting advice given to fisheries management authorities.

A second advantage of statistical age-structured models is that they provide an objective means of comparing model hypotheses regarding alternative "states of nature". In a maximum-likelihood framework, the usual frequentist approach of testing nested models using likelihood-ratio tests can be applied. In the Bayesian framework, the posterior odds of competing models can be computed. In either case, statistical guidance can be obtained regarding an appropriate model structure for the case at hand.

Both deterministic and statistical age-structured models rely on catch-at-age data. These are sometimes derived from the analysis of annuli on various body parts of individual fish. Perhaps more commonly, age composition is derived from length frequency samples using an age–length relation-

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ship prior to the age-structured analysis taking place. In this type of sequential approach, the variability in length-at-age is often ignored. It would be preferable to estimate age composition from the length frequency data and the parameters of the age-structured model simultaneously. In this way, parameter estimates would be conditioned on the length data rather than the catch-at-age estimates.

In this paper, we describe an age-structured model that extends the MULTIFAN method of estimating catch age composition from length composition (Fournier et al. 1990). The new model is called MULTIFAN-CL (Catch-at-Length). The major extension concerns the parameterization of the proportions-at-age in the length frequency samples. In the original MULTIFAN, the proportions-at-age are free parameters. Fournier et al. (1991) extended the model using a "survey sample" parameterization in which the length samples are assumed to be random samples of the population (with optional selectivity parameters for the initial age-classes). In MULTIFAN-CL, we assume that the length samples are taken from the catch of an age-structured fish population; the proportions-at-age in the length samples are therefore constrained by the catch equations that express the age-structured population dynamics. The model is fully integrated: growth and catch age structure are estimated simultaneously with recruitment, selectivity, catchability, natural mortality, and other parameters. Except for the parameterization of the proportions-at-age, the same likelihood function is employed for the length frequency data in all versions of the MULTIFAN model.

The Bayesian framework of the model is amenable to the formulation and testing of various hypotheses regarding the dynamics of the stock. Some of the model hypotheses that we formulate and test in this paper include spatial structuring of the population and fisheries, density-dependent growth, seasonal cycles in catchability, age-dependent rates of natural mortality, and age-dependent fish movement. The model is applied to catch, effort, and length frequency data for South Pacific albacore, *Thunnus alalunga*.

Data structures

The fundamental data structure of the model is a "fishery," which is defined as a collection of fishing units having similar catchability and selectivity characteristics with respect to the target species. Fisheries may be specific to geographical regions if spatial heterogeneity in the population and fisheries is to be modeled.

Each occurrence of a fishery at a particular time is termed a "fishing incident." In reality, fishing is more or less continuous, so the data for each fishery need to be aggregated over appropriate time intervals. Each fishing incident is associated with a data record, which is made up of an estimate of the total catch (in number of fish), the total effort, and a length frequency sample. Effort and the length frequency sample may be missing for some fishing incidents.

Catch equations

The catch equations govern the dynamics of the exploited age-structured population. To demonstrate the addition of spatial structure, we assume a one-dimensional, three-region spatial configuration. More complicated spatial structure could be accommodated within the model framework, as warranted by the particular application. For simplicity of notation, it is assumed that there is only one fishery operating in each region and that there is only one fishing incident per fishery per year. The model is easily generalized to accommodate a variable number of fisheries per region and fishing incidents per fishery per year.

The catch equations are as follows:

$$(1)\quad C_{ijk} = \frac{F_{ijk}}{Z_{ijk}}[1-\exp(-Z_{ijk})]N_{ijk} \quad \text{for } 1 \le i \le n,\ 1 \le j \le a,\ 1 \le k \le r$$

$$(2)\quad T_{i+1,j+1,k} = \exp(-Z_{ijk})N_{ijk} \quad \text{for } 1 \le i \le n,\ 1 \le j < a,\ 1 \le k \le r$$

$$(3)\quad T_{i+1,ak} = \exp(-Z_{i,a-1,k})N_{i,a-1,k} + \exp(-Z_{iak})N_{iak} \quad \text{for } 1 \le i < n,\ 1 \le k \le r$$

$$(4)\quad T_{i1k} = \gamma_k R_i \quad \text{for } 1 \le i < n,\ 1 \le k \le r \quad \text{where } \sum_k \gamma_k = 1 \text{ and } \gamma_k \ge 0$$

$$(5)\quad N_{ijk} = \sum_l \beta_{jkl} T_{ijl} \quad \text{for } 1 \le i \le n,\ 1 \le j \le a,\ 1 \le k \le r,\ 1 \le l \le r$$

$$(6)\quad Z_{ijk} = F_{ijk} + M_{ijk} \quad \text{for } 1 \le i \le n,\ 1 \le j \le a,\ 1 \le k \le r$$

$$(7)\quad C_{i\cdot k} = \sum_j C_{ijk} \quad \text{for } 1 \le i \le n,\ 1 \le j \le a,\ 1 \le k \le r$$

where i indexes year, j indexes age-class, k indexes region, n is the number of years of fishing, a is the number of age-classes in the population, r is the number of regions, C_{ijk} is the catch (in number of fish) of age-class j fish in region k in year i, $C_{i\cdot k}$ is the total catch observed in region k in year i, F_{ijk} is the instantaneous fishing mortality rate of age-class j fish in region k in year i, M_{ijk} is the instantaneous natural mortality rate of age-class j fish in region k in year i, Z_{ijk} is the instantaneous total mortality rate for age-class j fish in region k in year i, T_{ijk} is the number of age-class j fish in the population in region k at the beginning of year i before movement has taken place, N_{ijk} is the number of age-class j fish in the population in region k at the beginning of year i after movement has taken place, R_i is the recruitment at the beginning of year i, γ_k is the proportion of recruitment occurring in region k, and β_{jkl} is a k by k diffusion matrix $\mathbf{B}_j$ for age-class j fish.

The cumulative age-class (a) is designed to group fish above an age where they can be assumed to have insignificant growth (Fournier et al. 1991).

Movement hypothesis

The inclusion of spatial structure in the model requires the specification of a movement hypothesis. In the South Pacific albacore example, we use a one-dimensional diffusion model operating in three regions ($r = 3$). In this case, the elements of $\mathbf{B}_j$ are given by

$$(8)\quad \mathbf{B}_j = \begin{bmatrix} 1+v_j & -d_2 v_j & 0 \\ -v_j & 1+2d_2 v_j & -d_3 v_j \\ 0 & -d_2 v_j & 1+d_3 v_j \end{bmatrix}$$

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where 1, $d_2 > 0$, and $d_3 > 0$ specify the relative distribution of cohort abundance among regions at equilibrium and v_j is the age-dependent diffusion rate. We employ a flexible parameterization of v_j that can result in increasing, decreasing, or constant diffusion rate with increasing age:

$$(9) \quad v_j = \phi_0 \exp\{\phi_1[-(-\kappa_j)^{\phi_2}]\} \quad \phi_0 \geq 0, \phi_1 \geq 0, \text{ and } \kappa_j < 0$$

$$v_j = \phi_0 \exp\{\phi_1 \kappa_j^{\phi_2}\} \quad \phi_0 \geq 0, \phi_1 \geq 0 \quad \text{and } \kappa_j \geq 0$$

where $\kappa_j = \frac{2(j-1)}{a-1} - 1$, which expresses age scaled between −1 and 1.

Assumptions regarding constraints on natural and fishing mortality rates

A fundamental characteristic of statistical age-structured models is that they constrain the variation of mortality rates by age and time in a regular fashion. Constraints are placed separately on the variability of natural and fishing mortality rates.

Natural mortality

In the South Pacific albacore application, we assumed that the instantaneous natural mortality rate is independent of year and region, but may vary with age. Later, we show that this age dependency is supported by the data. For a given application, a range of more and less restrictive constraints on natural mortality can be tested.

Fishing mortality

We restrict the variation in the instantaneous fishing mortality rates F_{ijk} according to the "separability" assumption (Doubleday 1976; Paloheimo 1980; Fournier and Archibald 1982), which partitions F_{ijk} into an age-dependent effect (selectivity) and a time-dependent effect (catchability). Consider for simplicity an individual fishery (i.e., drop the k subscript). We assume that

$$(10) \quad \log(F_{ij}) = \log(s_j) + \log(q_i) + \log(E_i) + \varepsilon_i$$

$$(11) \quad \log(q_{i+1}) = \log(q_i) + \eta_i$$

where s_j is the selectivity for age-class j (assumed constant over time), q_i is the catchability in year i, E_i is the observed fishing effort in year i, ε_i are normally distributed random variables representing large transient deviations in the effort – fishing mortality relationship (or simply, effort deviations), and η_i are normally distributed random variables representing small permanent changes in catchability.

Selectivity can be modeled as a function of age-class, for example using a gamma function (Deriso et al. 1985). We have preferred to allow the s_j to be separate parameters but have applied a transformation that makes selectivity a length-based rather than an age-based concept (Appendix). The transformation ensures relatively small differences in s_j between adjacent age-classes having large overlap of their length distributions, as would be expected where selectivity is fundamentally length based.

Catchability is allowed to vary slowly over time. Following a concept introduced by Gudmundsson (1994), we assume that the q_i have the time series structure of a random walk (eq. 11), which is the simplest statistical model of a slowly varying random quantity. We make the prior assumption that the variance of η_i is small compared with ε_i.

Random walk steps can be taken at each successive fishing incident, or less frequently, as might be appropriate when multiple fishing incidents by one fishery occur within a year or fishing season. In the albacore analysis, random walk steps are taken annually for all fisheries.

Where the frequency of fishing incidents is greater than one per year, we may allow catchability within a year to vary with a regular seasonal pattern. Equation 10 then becomes

$$(12) \quad \log(F_{ij}) = \log(s_j) + \log(q_i) + \log(E_i) + c_1 \sin[24\pi(m - c_2)] + \varepsilon_i$$

where m is the month in which the fishing incident occurred and c_1 and c_2 are the seasonality parameters.

Assumptions regarding length-at-age

MULTIFAN-CL uses length data to estimate catch age composition and therefore makes assumptions concerning the length distribution of the fish. These assumptions are identical to those used in Fournier et al. (1990), to which the reader is referred for details. The assumptions are (*i*) the lengths of the fish in each age-class are normally distributed, (*ii*) the mean lengths-at-age lie on (or near) a von Bertalanffy growth curve, and (*iii*) the standard deviations of the lengths for each age-class are a linear function of the mean length-at-age.

We have introduced an additional, optional hypothesis concerning density-dependent growth, which can be incorporated into an analysis if warranted by the data. For many species, it is suspected that individuals of weak cohorts may grow faster than those of more abundant cohorts (i.e., density-dependent growth). If true, this phenomenon could have a large effect on the conclusions drawn from a length-based stock assessment.

Let R_β be the normalized relative cohort strength for cohort β, such that $R_\beta = (N_{\beta 1} - \bar{R})/\sigma_R$, where $\bar{R}$ and σ_R are the mean and standard deviation of recruitment. The changes in mean length are effected by adjusting the apparent age (i.e., the age implied from the length using the inverse of the growth function) of the fish before the length-at-age is calculated. For fish of cohort β, j years after recruitment, the adjusted age j' is

$$(13) \quad j' = j + 1.9\left[\frac{1}{1 + \exp(-gR_\beta)} - 0.5\right]$$

where g is a parameter determining the amount of density-dependent growth; if $g = 0$, $j' = j$. Since the standard deviation of R has been normalized to 1, the "generic" variation in R will be about −2 to 2. Thus the difference in j' between the strongest and weakest cohorts of any given age-class

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will be approximately $1.9\left[\frac{1}{1+\exp(-2g)} - \frac{1}{1+\exp(2g)}\right]$. For $g = -1.08$ (which is the estimate for the albacore data), this yields a generic variation of about –1.5 years, i.e., the adjusted age of the strongest cohort is about 1.5 years less than that of the weakest cohort.

Parameter estimation

Bayesian parameter estimation involves the computation of the mode of the posterior density function. We use the maximum of posterior distribution (MPD) method (Bard 1974), which involves maximizing the sum of the log-likelihood of the data plus the log of the prior density function. We therefore maximize a function consisting of the sum of three components: the log-likelihood of length frequency data, the log-likelihood of total catch estimates, and the log of the prior distribution for the effort – fishing mortality relationship.

Contribution of the length frequency data

Due to the large variability that often occurs in length frequency data, we employ a robust likelihood function. The motivation for using this procedure and its technicalities are described in Fournier et al. (1990). We shall not repeat this discussion here, but for convenient reference, we briefly describe the form of the log-likelihood function employed.

Let $\tilde{Q}_{i\alpha}$ denote the observed proportion of fish in length frequency sample α having a length lying in length interval i. If the $\tilde{Q}_{i\alpha}$ are derived from a random sample of size S_α, they would be random variables with means $Q_{i\alpha}$ and variances $(1 - Q_{i\alpha})Q_{i\alpha}/S_\alpha$. Two modifications have been made to this formula. If $Q_{i\alpha} = 0$, the formula implies that the variance of $\tilde{Q}_{i\alpha} = 0$. To decrease the influence of areas where no observations are expected, we add a small number to the variance formula. To reduce the influence of very large sample sizes, we have assumed that sample sizes >1000 are no more accurate than sample sizes of 1000. Set $\xi_\alpha = (1 - Q_{i\alpha})Q_{i\alpha}$ and $\tau_\alpha^2 = 1/\min(S_\alpha, 1000)$. Assume the variance of $\tilde{Q}_{i\alpha}$ is given by $(\xi_{i\alpha} + 0.1/I)\tau_\alpha^2$, where I is the number of length intervals in the length frequency samples. The likelihood function contribution for X length frequency samples is then

$$(14)\quad \prod_{\alpha=1}^{X}\prod_{i=1}^{I}\left[\frac{1}{\sqrt{2\pi(\xi_\alpha + 0.1/I)\tau_\alpha}} \times \left(\exp\left\{-\frac{(\tilde{Q}_{i\alpha} - Q_{i\alpha})^2}{2(\xi_{i\alpha} + 0.1/I)\tau_\alpha^2}\right\} + 0.01\right)\right].$$

Taking the logarithm of expression 14, we obtain the log-likelihood function for the length frequency data:

$$(15)\quad -1/2\sum_{\alpha=1}^{X}\sum_{i=1}^{I}\log\left[2\pi(\xi_{i\alpha} + 0.1/I)\right] - \sum_{\alpha=1}^{X} I\log(\tau_\alpha) + \sum_{\alpha=1}^{X}\sum_{i=1}^{I}\log\left[\exp\left\{\frac{-(\tilde{Q}_{i\alpha} - Q_{i\alpha})^2}{2(\xi_{i\alpha} + 0.1/I)\tau_\alpha^2}\right\} + 0.01\right].$$

The addition of 0.01 in eqs. 14 and 15 improves the robustness of the estimator by reducing the influence of observations that are more than about three standard deviations from the mean (Fournier et al. 1990).

Contribution of the observed total catches

Assuming for simplicity that there is only one fishery per year, the contribution of the observed total catches is given by

$$(16)\quad p_c\sum_i(\log(C_i^{obs}) - \log(C_i))^2$$

where p_c is determined by the prior assumption made about the accuracy of the observed catch data. For the albacore analysis, we assumed $p_c = 200$, which is consistent with a coefficient of variation of about 0.07.

Contribution of the prior distribution for the effort – fishing mortality relationship

Given the random walk structure assumed to operate for time-series changes in catchability, it follows that the prior distribution for the η_i is normal with zero mean. However, the prior distribution for ε_i is assumed to be a robustified normal distribution, i.e., the probability of events at the tails of the distribution is increased relative to a standard normal distribution. Then, the contribution of the Bayesian priors on the η_i and ε_i (see eqs. 10 and 11) is given by

$$(17)\quad p_\eta\sum_i\eta_i^2 - \sum_i\log\left[\exp(-p_\varepsilon\varepsilon_i^2) + 0.01\right].$$

The size of the constants p_η and p_ε is adjusted to reflect prior assumptions about the variances of η_i and ε_i. For the albacore analysis, we assumed $p_\eta = 25$ and $p_\varepsilon = 10$, which is equivalent to assuming that the coefficients of variation of η_i and ε_i are 0.14 and 0.22, respectively. Note that the second term of eq. 17 corresponds to an improper density. Therefore, the variance corresponding to the weight p_ε cannot be estimated and must be specified.

Nonlinear optimization

The parameters of the model are estimated by maximizing the sum of expressions 15, 16, and 17. The maximization was performed by an efficient optimization using exact derivatives with respect to the model parameters. The derivatives were calculated using an extension of the technique known as automatic differentiation (Griewank and Corliss 1991), an approach especially useful for models with large numbers of parameters. It also provides quick and accurate estimates of the Hessian matrix at the mode of the posterior distribution, which can be used to obtain estimates of the covariance matrix and confidence limits for the parameters of interest.

Estimation of confidence intervals

Confidence limits for the parameter estimates are calculated by employing the usual second-order approximation to the mode of the posterior distribution (Bard 1974). Let $\theta_1, \ldots, \theta_n$ denote a minimal set of n model parameters from which all model parameters can be calculated, and let $p(\theta_1, \ldots, \theta_n)$ be some parameter of interest, while $\ell(\theta_1, \ldots, \theta_n)$ is the logarithm of the posterior distribution. The estimated standard deviation, p_σ, for p is given by the square root of $\sum_{ij}\partial p/\partial\theta_i\partial p/\partial\Lambda_{ij}$,

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where $\Lambda_{ij} = (\partial^2 \ell / \partial\theta_i \partial\theta_j)^{-1}$, with the calculations carried out at the MPD. Then, 95% confidence limits for the p are given by $[p - 1.96p_\sigma, p + 1.96p_\sigma]$. These confidence limits are not invariant under reparameterization. To compensate somewhat for this, the confidence limits for parameters that must be positive, such as estimates of biomass, are calculated by computing the confidence limits for the logarithms of these parameters and then transforming the confidence limits. This yields the confidence limits $[p \exp(-1.96\, p_\sigma/p),\ p \exp(1.96\, p_\sigma/p)]$.

The above procedure provides approximate confidence intervals for the model parameters (initial cohort size, selectivity and catchability coefficients, natural mortality rates, growth parameters, etc.). For stock assessment purposes, it may be desirable to have confidence intervals for quantities of interest, such as adult biomass, that are functions of the model parameters. The variances (and hence, confidence intervals) for such quantities are determined using the delta method.

Hypothesis testing

It is frequently of interest in statistical modeling to add model structure in the form of a hypothesis concerning some process of interest and to observe the resulting change in model performance. Let H_1 (with n_1 parameters) and H_2 (with n_2 parameters) denote alternative models to be tested. In a Bayesian framework, the support for H_2 over H_1 provided by the data D is measured by the posterior odds, which is the product of the ratio of the integrated likelihoods, known as the Bayes factor (Kass and Raftery 1994), and the prior odds for H_2 against H_1:

$$(18) \qquad \frac{p(H_2|D)}{p(H_1|D)} = \left[\frac{p(D|H_2)}{p(D|H_1)}\right]\left[\frac{p(H_2)}{p(H_1)}\right].$$

The Bayes factor is interpreted as the sample "weight of evidence" for H_2 over H_1.

While appealing on theoretical grounds, there are two impediments to applying Bayes factors in practice. First, it is necessary to provide a Bayesian prior distribution for the parameters. For large problems the usual practice is to provide specific Bayesian priors for a small number of parameters of interest and to apply a locally uniform prior on the remaining parameters. This causes no problem when computing point estimates of the parameters. However, when computing Bayes factors, such diffuse priors lead to the well-known Lindley paradox (Aitkin 1991) where, for point null hypotheses, the Bayes factor will tend to infinity as sample size tends to infinity.

A second difficulty is that evaluation of the Bayes factor involves integration of the likelihood functions over all model parameters. For large models (such as the albacore model), this may not be feasible because of limited computing power.

To avoid these problems, we have employed *posterior* Bayes factors (Aitkin 1991), which allow (but do not require) models to include priors with diffuse specifications. Posterior Bayes factors are generally applicable for arbitrary models and their use does not lead to the Lindley paradox (Aitkin 1991). The asymptotic form for the posterior Bayes factor for H_2 over H_1 is

$$(19) \qquad A_{21} = \frac{L_2(\theta_2)}{L_1(\theta_1)}\, 2^{(n_1 - n_2)/2}.$$

This is a penalized version of the likelihood ratio, with the penalty based on the difference in the number of parameters of the two models. The A_{21} is then a measure of the weight of sample evidence in favor of H_2 over H_1. We use Aitkin's (1991) calibration that values of A less than 0.05, 0.01, and 0.001 constitute "strong," "very strong," and "overwhelming" sample evidence, respectively, of one model over the other.

Application to South Pacific albacore

Background

Albacore comprise a discrete stock in the South Pacific Ocean (Murray 1994). Adults (larger than about 80 cm fork length) spawn in tropical and subtropical waters between about 10 and 25°S during the austral summer (Ramon and Bailey 1996), with juveniles recruiting to surface fisheries in New Zealand coastal waters and in the vicinity of the subtropical convergence zone (STCZ) in the central Pacific about 2 years later. Distant-water longline fleets of Japan, Korea, and Taiwan and domestic longline fleets of several Pacific Island countries catch primarily adult albacore virtually throughout their range. A troll fishery for juvenile albacore has occurred in New Zealand coastal waters since the 1960s and in the central Pacific in the region of the STCZ since the mid-1980s. Driftnet vessels from Japan and Taiwan targeted albacore in the central Tasman Sea and in the central Pacific near the STCZ during the 1980s. Surface fisheries are highly seasonal, occurring mainly during December–April, while longline fisheries operate throughout the year. Total annual catches have varied between 20 000 and 52 000 t since the 1960s. Longline gear accounts for the majority of the catch, about 30 000 t·year^{-1} on average. Troll catches are relatively small, generally producing less than 10 000 t·year^{-1}. The driftnet catch reached 22 000 t in 1989, but has since declined to zero following a United Nations moratorium on industrial-scale driftnetting. The approximate distribution of the fisheries is shown in Fig. 1.

Fisheries data

Catch, effort, and size composition data have been routinely collected from the fisheries since the early 1960s. The data have inconsistent temporal resolution and include periods where effort, length frequency data, or both are missing. Length frequency sample sizes are highly variable. Such heterogeneous data, which are typical of many fisheries data sets, are readily handled by MULTIFAN-CL.

We defined fisheries on the basis of fishing method and region. Three regions are specified by the latitudinal bands 0–10°S (region A), 10–30°S (region B), and 30–50°S (region C) (Fig. 1). The distant-water longline fleets fishing in these regions are defined as separate fisheries by region, but are aggregated across nationality. The fleets of small-scale, domestic longliners that have developed in several Pacific Island countries (region B) in recent years are also defined

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Fig. 1. Distribution of longline (light shading), troll (medium shading), and driftnet (dark shading) fisheries for South Pacific albacore. The three rectangular zones define the model regions, which are used in the classification of fisheries and spatial stratification of the model.

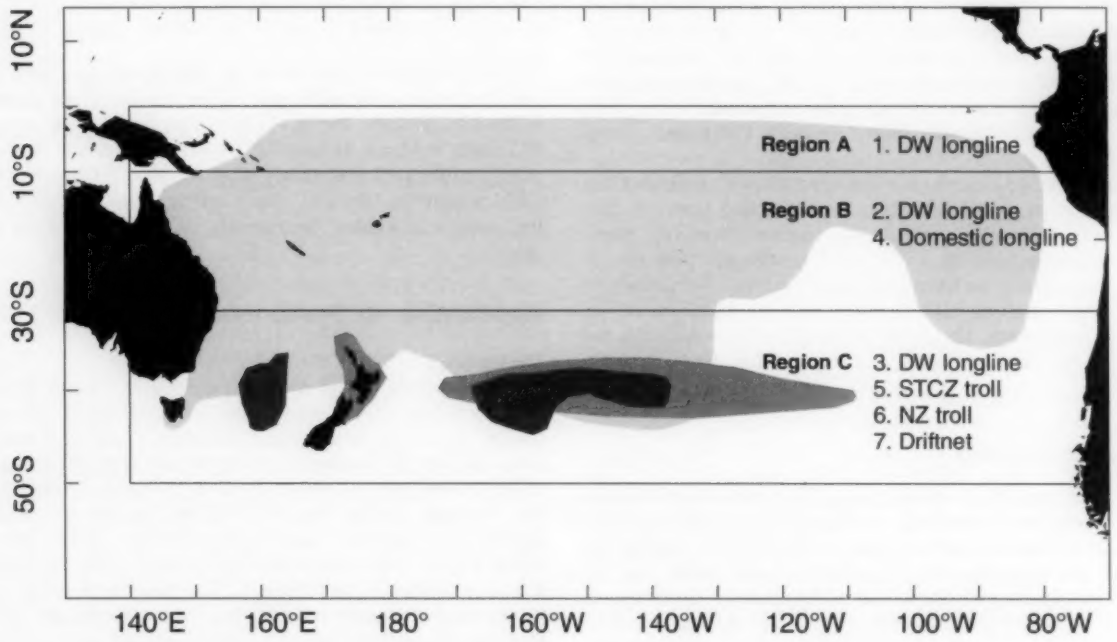

Table 1. Posterior Bayes factors (A_{i1}) for comparisons of model 1 with model i (i = 2, ..., 6).

Model	Log-likelihood	No. of parameters	A_{i1}
1. Full model	57 543.7	901	
2. Model 1 without spatial structure	57 268.4	876	1.6×10^{-116}
3. Model 1 with age-independent diffusion	57 491.9	899	6.38×10^{-23}
4. Model 1 with age-independent natural mortality	57 519.7	892	8.54×10^{-10}
5. Model 1 without seasonal catchability for all fisheries	57 127.0	887	1.4×10^{-179}
6. Model 1 without density-dependent growth	57 430.3	900	7.97×10^{-50}

as a fishery. Other fisheries are the troll fishery in New Zealand coastal waters, the troll fishery operating in the STCZ, and the driftnet fishery (all region C).

For the longline fisheries, fishing incidents are aggregated by quarterly time periods (January–March, April–June, July–September, October–December). For the surface fisheries, which tend to operate during the summer months only, fishing incidents are aggregated by month. With this data stratification, the albacore database for years 1962–1993 consists of 591 fishing incidents. Of these, 566 fishing incidents have a fishing effort estimate and 381 have a length frequency sample.

Constraints and model hypotheses:

It is necessary to specify the number of age-classes to be considered in the model. For the presentation of results below, we chose a model with 11 age-classes, which is consistent with previous aging studies (Labelle et al. 1993). Trials with larger numbers of age-classes did not significantly alter the results of the analysis.

We applied a one-dimensional diffusive movement hypothesis to the three-region spatial structure of the model. This hypothesis was considered to be a reasonable, albeit simple, representation of South Pacific albacore movement on the basis of qualitative examination of tagging data (Labelle 1993) and the variation in albacore size with latitude (smallest in the south, increasing towards the equator). Other movement hypotheses and (or) spatial configurations could easily be incorporated into the model and tested. One such alternative hypothesis (spatial homogeneity) has been tested and is discussed below.

Several additional constraints on the model were necessary to produce stable behavior during parameter estimation. These include constraints that recruitment occurs only in region C (the southernmost region), recruitment varies lognormally among years, and selectivity coefficients are constant over time within fisheries.

The hypothesis testing procedure outlined earlier was used to test numerous alternative model structures, consisting of various combinations of the following model hypoth-

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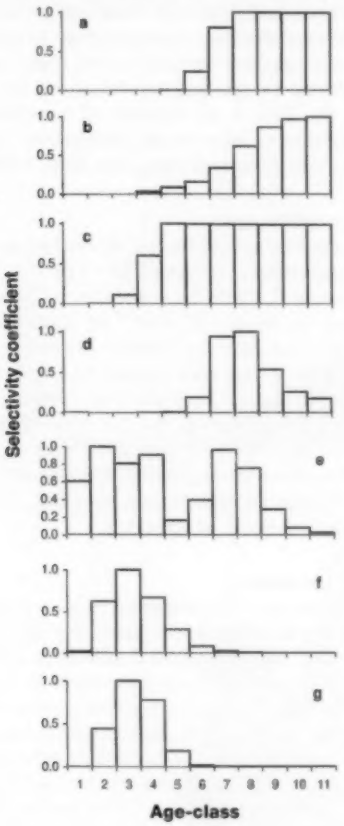
Fig. 2. Estimated South Pacific albacore selectivity coefficients. Panels *a*–*g* correspond to fisheries 1–7 as shown in Fig. 1.



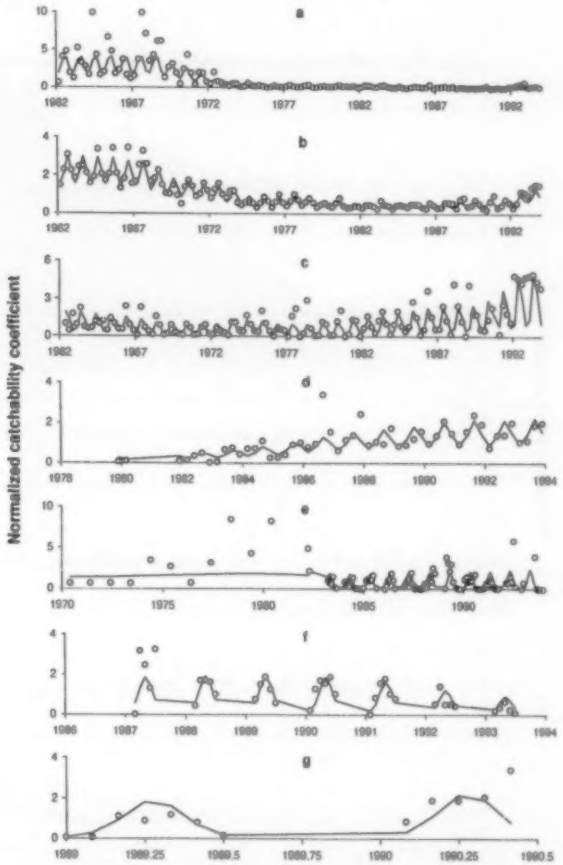
eses (with the simpler alternative hypothesis in parentheses): spatial structuring of the population into three regions, as defined earlier (spatial homogeneity), age-dependent (age-independent) diffusion of fish among regions, age-dependent (age-independent) natural mortality rate, seasonal variability (constancy) in catchability for all fisheries, and density-dependent (density-independent) growth. The posterior Bayes factors indicated overwhelming support of the data for each of these hypotheses (Table 1). While these tests are by no means exhaustive, they indicate how hypotheses that are supported by the data can be readily identified and incorporated into the analysis.

Some of the results of the South Pacific albacore analysis that illustrate the model features are given below.

Selectivity and catchability coefficients

Estimated selectivity coefficients (Fig. 2) broadly reflect differences in catch size composition among the fisheries. Most fisheries display a regular pattern of selectivity, either increasing with age-class or unimodal over a restricted range of age-classes. In contrast, the New Zealand troll fishery shows a bimodal selectivity pattern, probably reflecting some contamination of this data set with data from different fishing methods.

Fig. 3. Estimated South Pacific albacore catchability coefficients (normalized to the average for each fishery) (solid lines) and deviations from the effort – fishing mortality relationship (open circles). Panels *a*–*g* correspond to fisheries 1–7 as shown in Fig. 1.



Estimated catchability time series are plotted with effort deviations (ε_i), by fishery, in Fig. 3. Strong trends in catchability are estimated for some of the fisheries. For the longline fisheries, this is likely to be due to changes in species targeting by the fleets concerned. Strong seasonal variation, which was a highly significant addition to the model (Table 1), is also evident for all fisheries. This seasonality is possibly related to changes in the vertical distribution of the albacore in response to seasonal temperature variation. After the removal of time series trends and seasonal variation in catchability, the effort deviations are mostly evenly distributed about zero (Fig. 4), indicating that there is no further information in the data regarding catchability variation.

Growth parameters and catch age composition

The estimation of catch age composition from length composition assumes, amongst other things, that albacore exhibit von Bertalanffy growth. We use a parameterization such that growth is specified by three parameters: the mean length of the first age-class (45.3 cm), the mean length of the last age-class (100.9 cm), and growth coefficient K

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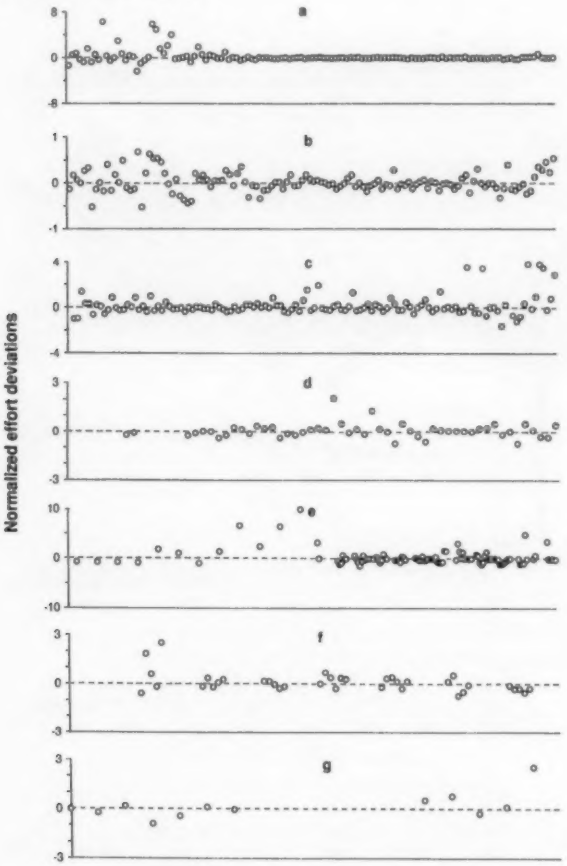
Fig. 4. Deviations from the effort – fishing mortality relationship after removal of time series trends and seasonal variation in catchability. The deviations are normalized to the average catchability for each fishery. Panels *a–g* correspond to fisheries 1–7 as shown in Fig. 1.



(0.190·year^{-1}). These parameters can be transformed to provide the usual von Bertalanffy growth parameter L_∞ (107.2 cm). Estimated growth rates over the range of exploited sizes are almost identical to estimates based on vertebral ring counts (Labelle et al. 1993).

There is good correspondence between estimated mean lengths-at-age and obvious modes in the length frequency samples for most of the fisheries. Examples of the fits to the length frequency data are shown in Fig. 5.

The addition of density-dependent growth to the model makes the growth of individual cohorts dependent on their initial relative abundance. This effect appears to be quite strong in South Pacific albacore, with more abundant cohorts growing slower than less abundant cohorts — the difference in apparent age between the largest and smallest cohorts is about 1.5 years. The effect of density-dependent growth on the estimated mean lengths-at-age is shown in Fig. 5 by the differences between the solid and broken vertical lines for each age-class.

Exploitation rates

Exploitation rates (the proportion of the population harvested per year) and their 95% confidence intervals for two age groups, corresponding approximately to age-classes 2–5 (primarily exploited by the surface fisheries) and 6–11 (primarily exploited by the longline fisheries), have been computed (Fig. 6). This is an example of quantities of interest that are functions of other model parameters, with their confidence intervals estimated using the delta method.

Natural mortality rates

The inclusion of age-dependent M resulted in a highly significant improvement in model fit (Table 1). The M estimates are about 0.2·year^{-1} for age-classes 1–5, after which they increase to about 0.35·year^{-1} by age-class 9 (Fig. 7). The point at which M is estimated to increase (at about age-class 6, or about 85 cm fork length) corresponds well to the size at onset of female reproductive maturity (Ramon and Bailey 1996). At this point, the sex ratio of adult albacore also changes rapidly with increasing size to favor males (Fig. 7). This raises the possibility that M may be greater for older fish because of high female mortality associated with the physiological stress of spawning.

Movement parameters

The diffusion rate was estimated to be a decreasing function of age, declining from 0.35·year^{-1} for age-class 1 to less than 0.10·year^{-1} for age-classes 5 and older. The confidence intervals on the estimates are equivalent to coefficients of variation of about 0.7, suggesting that there is limited information on the catch, effort, and length frequency data on movement. Nevertheless, age-dependent diffusion was a significant model hypothesis (Table 1). Net movement of albacore occurs from south to north. The estimates of population biomass indicate that most of the stock is located in regions B and C, with very little located in the northernmost region (Fig. 8). Ignoring spatial structure entirely resulted in a highly significant degradation in model fit (Table 1).

Population biomass and recruitment

The time series of population biomass and recruitment are key outputs of the model from a stock assessment viewpoint. The biomass estimates (Fig. 8) show a strongly increasing trend up to the mid-1970s and a decreasing trend thereafter until about 1990. The trends are similar in the three regions.

The recruitment estimates (Fig. 9) are higher for the first half of the time series and lower and more variable during the second half. This pattern drives the biomass trends observed in Fig. 8. Relatively low estimates of recruitment are obtained for 1980, 1985, and 1990. Assuming that the age of recruitment is about 2 years (consistent with the Labelle et al. (1993) vertebral ring count estimates), the spawning seasons corresponding to the low recruitments match well with the occurrence of El Niño episodes (negative values of the Southern Oscillation Index) in the Pacific Ocean (Fig. 9). The high recruitments in the second half of the time series also correspond to La Niña events (positive values of the Southern Oscillation Index) 2 years prior to recruitment. The relationship is not as good over the first half of the time series, probably because the absence of fisheries directed at

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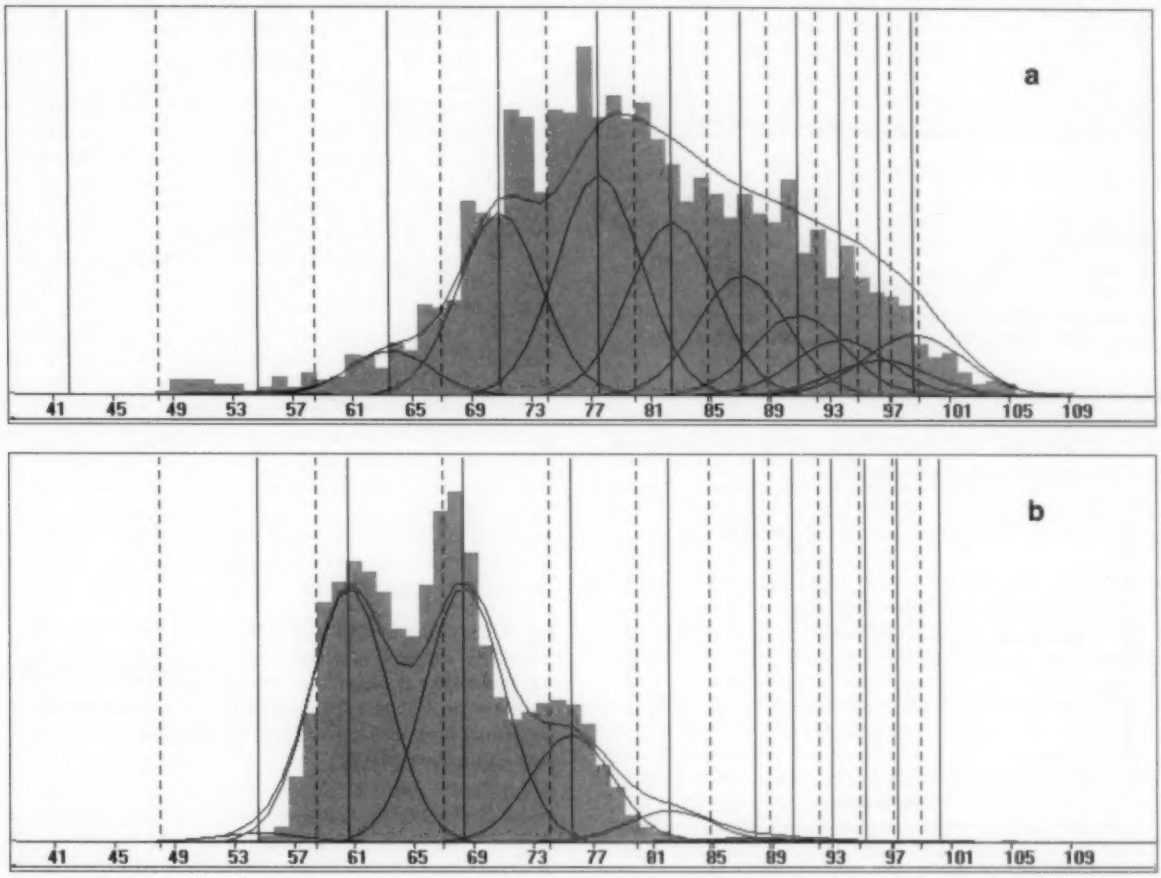
Fig. 5. Examples of fits (model 1, Table 1) to the South Pacific albacore length frequency data. The solid vertical lines indicate estimated mean lengths-at-age. The broken vertical lines indicate mean lengths-at-age in the absence of density-dependent growth. Both the estimated aggregate (upper line) and age-class specific length distributions are shown. (*a*) Sample from the distant-water longline fishery in region C (second quarter, 1973); (*b*) sample from the subtropical convergence zone troll fishery in region C (March 1990).



small albacore during this period results in less informative data for estimating recruitment variability.

Discussion

We have developed an integrated, age-structured model using length data that may, for many fisheries, be a viable alternative to sequential methods. Unlike most age-structured models that use length data to estimate catch age composition, our approach fully integrates catch age composition and growth parameter estimation with estimation of recruitment, mortality, and related parameters of the age-structured model.

The model should be potentially applicable to fish stocks that exhibit an age-class signal in length frequency data. Extensive simulation trials during early model development indicated that, as expected, the precision of parameter estimates erodes as the variability of length-at-age increases. Where there is little information on catch-at-age in the length frequency data, the incorporation of independent age–length observations into the estimation as auxiliary data may help resolve catch-at-age estimation.

Simulation trials have also confirmed the obvious result that catchability trends will tend to be underestimated if the mean of the prior distribution for the η_i is zero. Nevertheless, model performance is much better than if constant catchability was assumed. In the case of the albacore analysis, the strong catchability trends estimated for the distant-water longline fisheries are suspected to be due at least in part to changes in targeting practices among the various national fleets. Further stratification of the longline data, or standardization to reduce the effects of targeting variability, may therefore be warranted.

The statistical approach to the age-structured model and Bayesian framework for parameter estimation and hypothesis testing offers the advantage of being able to objectively assess the information content of the data. This enables the construction of approximate confidence intervals on the parameters of interest. Because the model is fully integrated, such confidence intervals incorporate uncertainty arising from the estimation of catch-at-age from length data, as well

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Fig. 6. Estimated average annual exploitation rates (thick lines) and their 95% confidence intervals (thin lines) for (*a*) combined age-classes 2–5 and (*b*) combined age-classes 6–11.

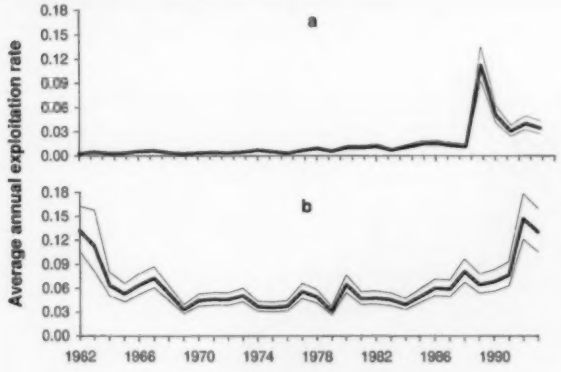
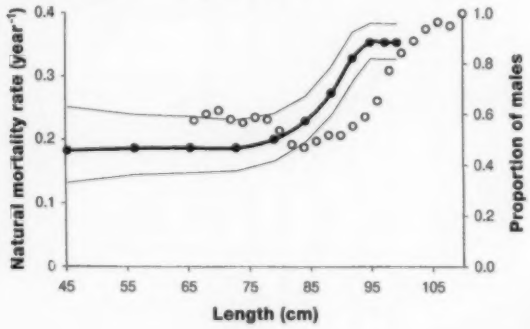


Fig. 7. Estimates of South Pacific albacore age-specific natural mortality rates (solid circles, thick line), their 95% confidence intervals (thin lines), and observed proportions of male albacore by 2-cm length-classes (open circles).



as other sources of variability. The statistical approach also allows testing of alternative model hypotheses, using posterior Bayes factors, enabling sensible decisions to be made regarding model development.

We have adopted two variations on classical Bayesian methods regarding estimation of confidence intervals and hypothesis testing. First, we assume that the posterior distribution is approximately normal in the vicinity of its mode and that the covariance matrix is equal to the inverse of the Hessian. These assumptions are standard in maximum-likelihood estimation and are reasonable in the Bayesian context for problems with largely noninformative priors (Bard 1974). Second, we use posterior Bayes factors rather than the posterior odds for hypothesis testing. In both cases, our objective was to avoid the necessity of numerically intensive, high-dimensional integration of the posterior density function. While Monte Carlo methods such as the sampling-importance resampling (SIR) and the Monte Carlo Markov Chain (MCMC) algorithms provide efficient means for approximating such integrals (McAllister and Ianelli 1997), they are still not feasible for models the size of the albacore model presented in this paper.

Although further questions concerning the albacore analysis need exploration, the consistency of the model results with various exogenous data sets is encouraging. These include the consistency of MULTIFAN-CL length-at-age estimates with those previously derived from vertebral ring counts, the consistency of age-dependent natural mortality rate estimates with changes in albacore sex ratio with size and with the size at onset of female reproductive maturity, and the apparent relationship between variation in estimated recruitment and variation in the Southern Oscillation Index. While these relationships require further study, their persistence in ongoing analyses of updated albacore fisheries data would provide convincing validation of the model results. In

Fig. 8. Estimated relative (scaled to the average) biomass of South Pacific albacore (thick lines) with 95% confidence intervals (thin lines) in (*a*) region A (0–10°S), (*b*) region B (10–30°S), and (*c*) region C (30–50°S).

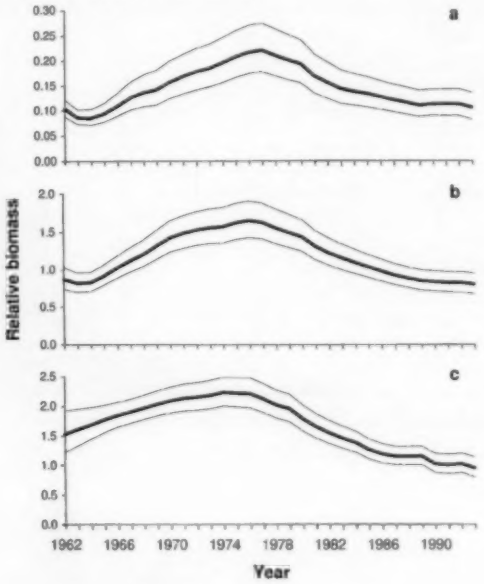
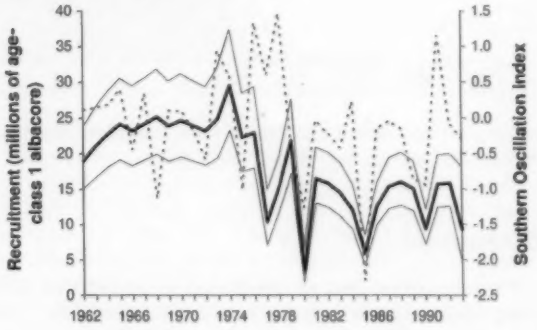


Fig. 9. Estimated South Pacific albacore relative recruitment (thick line) and 95% confidence intervals (thin lines) by year. The broken line is the average annual Southern Oscillation Index 2 years prior to recruitment. Negative values of the Southern Oscillation Index indicate El Niño episodes and positive values indicate La Niña episodes.



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this case, formal incorporation of such exogenous data into the model as auxiliary data could improve the precision and predictive power of the model.

The model could be a useful tool for management of the South Pacific albacore and other fisheries. Two key uses come readily to mind and would require only minimal adaptation of the existing computer software. First, the results of the model could be cast in a form suitable for comparison with limit or target reference points, as envisaged by the recent United Nations agreement on straddling and highly migratory fish stocks (Levy and Schram 1996). This could be done by calculating the probability that a chosen reference point is violated by the present estimated population state or by projected future population states estimated under a particular fishing regime. In the latter case, such forward projections would require a model for future recruitment (perhaps linked to large-scale environmental conditions such as indicated by the Southern Oscillation Index). This and other fishery performance measures provide a convenient framework for incorporating uncertainty in the assessment into management advice (Punt and Hilborn 1997). Second, forward projections could be a useful short-term forecasting tool for both the surface and longline fisheries, particularly if the predictability of recruitment from environmental variables is confirmed. Confidence intervals could be determined for the projections to capture the uncertainty in future recruitment and the current population state. Such forecasting could assist both industry and management decision-making.

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Appendix. Length-based selectivity coefficients

This appendix describes the transformation of age-based selectivity coefficients to coefficients that account for the degree of length overlap of age-classes. The transformation is designed to make the selectivity coefficients reflect a

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length-based rather than an age-based process. The following symbols are used in the derivation of the length-based selectivity coefficients: c_j is the "age-based" selectivity coefficient for age-class j fish; s_j is the "length-based" or length-averaged selectivity for age-class j fish; ω_k are weights, determined from the normal distribution of length-at-age, k standard deviations from the mean; μ_j is the mean length of age-class j fish; σ_j is the standard deviation of length of age-class j fish; and L_1, L_a, and ρ are the von Bertalanffy growth parameters.

Let $\phi(L_1, L_a, \rho)$ denote the von Bertalanffy growth function, so that $\phi^{-1}(\mu_j) = j$. Let $\phi^{-1}(\mu_j + \sigma_{k\sigma_j}) = i_{jk} + x_{jk}$, where i_{jk} is an integer, $0 \le x_k < 1$, and δ_k is the kth component of the vector (–1, –0.5, –0.25, 0, 0.25, 0.5, 1). Then:

$$\text{(A1)} \quad s_j = \sum_{k=-3}^{3} \omega_{|k|}\{c_{i_{jk}}(1 - x_{jk}) + c_{i_{jk}+1}x_{jk}\}$$

where $\omega_k = w_k/(w_0 + 2w_1 + 2w_2 + 2w_3)$, $w_0 = 1$, $w_1 = \exp(-0.25^2/2)$, $w_2 = \exp(-0.5^2/2)$, and $w_3 = \exp(-1.0/2)$.

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Assessing the impacts of acidification on Atlantic salmon (*Salmo salar*): a simple model of stream chemistry

D.R. Marmorek, G.L. Lacroix, J. Korman, I. Parnell, and W.D. Watt

Abstract: We developed a model that simulates the effects of changes in sulphate (SO_4^{2-}) deposition on the chemistry of naturally organic-rich streams, linked this chemical model to a model of Atlantic salmon (*Salmo salar*) production (Korman et al. 1994. Can. J. Fish. Aquat. Sci. **51**: 662–680), and assessed its performance on three acidified streams in southwest Nova Scotia. The chemical model closely tracked current chemistry by estimating the charge density required for charge balance on each sampling date. Calculated charge densities were generally low (1–3 μequiv./mg dissolved organic carbon (DOC)), inversely related to DOC, and positively related to pH. Predictions of minimum pH and salmon smolt output were relatively insensitive to the assumed *F*-factor (watershed neutralization of deposited acidity) in the parameter range most likely for the three streams. The model permits rapid impact assessment of acid deposition scenarios with a modest amount of input data (acid-neutralizing capacity, pH, SO_4^{2-}, and DOC, ideally sampled weekly) while retaining natural cycles and processes.

Résumé : Nous avons mis au point un modèle qui simule les effets des changements dans les dépôts de sulfate (SO_4^{2-}) sur la chimie des cours d'eau naturellement riches en matière organique, lié ce modèle chimique à un modèle de la production de saumon atlantique, *Salmo salar* (Korman et al. 1994. J. can. sci. hal. aqu. **51**: 662–680), et évalué sa performance dans trois cours d'eau acidifiés du sud-ouest de la Nouvelle-Écosse. Le modèle chimique suivait de près la chimie réelle en estimant la densité de charge nécessaire pour l'équilibre à chaque date d'échantillonnage. Les densités de charge calculées étaient généralement faibles [1–3 μéquiv./mg de carbone organique dissous (COD)], inversement reliées au COD et positivement reliées au pH. Les prédictions du pH minimum et de la production de smolts étaient relativement insensibles au facteur *F* présumé (neutralisation par le bassin versant de l'acidité déposée) dans la fourchette de paramètres la plus vraisemblable pour les trois cours d'eau. Le modèle permet d'évaluer rapidement l'impact de divers scénarios de dépôts acides avec une quantité modeste de données d'entrée (capacité de neutralisation de l'acide, pH, SO_4^{2-} et COD, avec, dans des conditions idéales, un échantillonnage hebdomadaire) en se fondant sur les cycles et processus naturels.

[Traduit par la Rédaction]

Introduction

The acidification of rivers in southwest Nova Scotia has been associated with significant losses of Atlantic salmon (*Salmo salar*) (Lacroix 1987). This acidification is due to the additive effects of anthropogenic deposition of mineral acids and watersheds' natural organic acids, resulting in both chronic and episodic acidic conditions (Kerekes et al. 1986). Watt (1987) suggested that anthropogenic acids may have been responsible for a 50% decrease in salmon production, although his estimates (based on juvenile salmon densities in streams with different pH levels) were very preliminary. Questions remain regarding the actual historical losses of salmon production and the adequacy of recent reductions in S emissions and deposition for initiating recovery. Fishery scientists and managers need to better understand the potential range of responses in acidified, organic streams of Nova Scotia to future deposition changes and their effects on salmon populations.

We have built some tools to explore possible answers to these questions and to assess the uncertainty in these answers. Our strategy was first to build and apply a biological model consistent with field acidification experiments (Atlantic salmon regional acidification model (ASRAM)) and explore its sensitivity to variations in stream chemistry (Korman et al. 1994; Lacroix and Korman 1996). In this paper, we describe a simple model for translating the current chemistry of a stream into the chemistry it would exhibit under a different level of acidic deposition. In building this model, we attempted to satisfy three criteria. First, the chemical model needed to link into ASRAM, so that we could predict the impacts of changes in acidic deposition on Atlantic salmon. Second, limited existing stream chemistry data meant that the model should have modest input data requirements to be able to answer regional-scale questions. Third, to be scientifically credible, the model must also simulate

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Table 1. Summary of stream conditions.

Stream	pH		DOC (mg/L)		ANC (μequiv./L)		SO_4^{2-} (μequiv./L)		C_B (μequiv./L)	FM × 100 (%)	Ion ratio used
	Mean	Range	Mean	Range	Mean	Range	Mean	Range			
Westfield River	5	4.7 to 5.4	9.3	6.4 to 16.1	2.5	−14.0 to 12.0	72.2	56.2 to 85.4	61.5	17.4	Na/Cl
Round Lake Brook	4.7	4.4 to 5.0	12.7	9.9 to 22.6	−6.8	−26.0 to 8.0	77.3	62.5 to 99.9	62.9	18.1	Na/Cl
Moose Pit Brook	4.6	4.4 to 5.0	20.5	7.8 to −43.2	−9.3	−28.0 to 14.0	72.1	43.7 to 131.2	73.2	19.6	Na/Mg

Note: Measured pH and DOC; C_B (total nonmarine base cations) estimated from stream measurements and seasalt correction equations from Thompson (1982*a*) and FM × 100 (% marine SO_4^{2-}) estimated from ion ratios in last column using Vet et al. (1986). Na/Mg used for Moose Pit Brook because it provided more accurate corrections than Na/Cl.

short-term acidic episodes in organically enriched streams, a key driving force in the biological responses of Atlantic salmon (Lacroix and Korman 1996).

Existing models that operate on daily or weekly time scales (e.g., Lam et al. 1989) are calibrated using detailed flow and chemistry measurements over several years (Cosby et al. 1985). Such detailed data sets are rare. For example, there are 63 rivers in the acidified Southern Upland region of Nova Scotia that once supported Atlantic salmon (Watt 1987), and each of these rivers has many tributary streams capable of supporting salmon. However, there are only about 10 streams in that region with measurements of pH, alkalinity (ALK), dissolved organic carbon (DOC), and sulphate (SO_4^{2-}) on a monthly or greater frequency. Our chemical model has fewer input data requirements than most acidification models while maintaining the ability to accurately simulate short-term changes in stream chemistry. While simplification creates some limitations, we consider the model to be a generally applicable tool for organic, acid-sensitive streams, although region-specific calibration and validation should be completed before it is applied outside southwest Nova Scotia.

The model was developed in VisualBasic™ and operates under the Windows™ environment on IBM PC compatible computers, permitting easy adjustment of parameters and Monte Carlo simulations across a range of deposition scenarios and parameter values. (The software and limited documentation are available from Dr. G.L. Lacroix for noncommercial use only (please send a high-density diskette).)

Methods

General modelling approach

Previous work has shown the importance of seasonal changes in wetland retention of mineral and organic acids on the seasonal patterns of chemistry in Nova Scotia streams (e.g., Kerekes and Freedman 1989). We took a modelling approach that would easily *translate* current measured stream ALK, SO_4^{2-}, and pH levels into a new time series, without having to calibrate many equations representing watershed hydrologic and chemical processes. We preserved the seasonal pattern of DOC but changed SO_4^{2-} concentration ($[SO_4^{2-}]$) in response to changes in deposition, and shifted ALK, organic anions, and pH appropriately, based on simple equilibrium calculations. We therefore called this the time series translation model (TSTM). Its output represents the seasonal pattern of pH values once a stream has come to steady state with a new deposition value. The model does not estimate how long it will take for the stream to reach this steady state.

TSTM assesses the current condition of a stream and then projects its future condition (Fig. 1). The assessment of current condition is a calibration to estimate the charge densities that are consistent with the ALK, pH, and DOC measured on each sample date. These charge densities are then used in the projection of future conditions in the stream after the ALK has been adjusted to reflect changes in mineral acids (bottom of Fig. 1). These steps are described below.

Inputs

The data inputs for TSTM are simply (*i*) measurements of stream Gran ALK, pH, $[SO_4^{2-}]$, and DOC measured at a time resolution necessary to capture major seasonal fluctuations and lowest pH values (i.e., ideally weekly during the fall and winter and bi-

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weekly to monthly at other periods) and (*ii*) an estimate of the proportion of $[SO_4^{2-}]$ that is from marine sources (requiring at least stream measurements of Cl^- and preferably also Ca^{2+}, Na^+, and Mg^{2+}). We used Vet et al. (1986) to estimate marine $[SO_4^{2-}]$; their method compares the ratios of Na^+/Mg^{2+}, Na^+/Cl^-, and Mg^{2+}/Cl^- in water samples with the ratios in seawater and chooses the "best" tracer. To simulate a deposition scenario, the user specifies a fractional change in the amount of anthropogenic SO_4^{2-} deposition, relative to the deposition levels associated with the measured time series of stream chemistry. We chose to apply TSTM to three streams of the Medway River, Nova Scotia, for which frequent (i.e., weekly) measurements of water chemistry were available and that spanned a range of pH and DOC levels: Westfield River, Round Lake Brook, and Moose Pit Brook (Table 1). Characteristics and hydrology of the drainage basin for the streams were described by DeGraeve and Peterson (1982). Chemical parameters were measured using standard methods as described in Lacroix and Kan (1986) and Lacroix and Townsend (1987). The dominance of nonexchangeable forms of Al in these organic streams makes pH the main lethal factor directly affecting survival and production of juvenile salmon (Lacroix 1989) and also permits us to ignore Al^{n+} in our charge balance equations.

The biological model (ASRAM) runs from June 1 to May 31 and requires chemical data over this same period. In order to meet this input sequence, we shuffled the order of the annual time series of data (March 1, 1983, to February 28, 1984) for the three streams. The modelled periods were therefore from June 1, 1983, to February 28, 1984, and then from March 1, 1983, to May 31, 1983. This reordering of the time series did not generate any unrealistic discontinuities at the break points.

The parameter assumptions required for the model are (*i*) an estimate of the *F*-factor (Wright and Henriksen 1983) to reflect watershed neutralization of mineral acid deposition, (*ii*) the pCO_2 level, (*iii*) the proportion of SO_4^{2-} that is of marine origin, and (*iv*) functions controlling organic dissociation (either the approach of Oliver et al. (1983) or specified dissociation constants, pK_as). Charge density (CD_t) can be either measured (consistent with the definition below) or estimated by the model from water chemistry data to maintain charge balance for each sampling date. We use the latter approach (dynamic CD_t calculation), as it allows for the best simulation of current seasonal patterns in pH levels. We define CD_t as micoequivalents of total organic anion per milligram of DOC, if all anions are dissociated. We later adjust for the pH-dependent degree of dissociation (eq. 6 below). Total organic anions (A^-) include A^{3-}, HA^{2-}, and H_2A^-.

Model structure

The model is founded upon the following charge balance equation (Stumm and Morgan 1981):

$$(1) \qquad \text{Gran ALK} = HCO_3^- + CO_3^{2-} + OH^- + A^- - H^+$$

where A^- are dissociated organic anions and all chemical species are expressed in microequivalents per litre. Both CO_3^{2-} and OH^- are close to zero at the pH levels observed in the three streams we modelled. As stated previously, we assume that $Al^{n+} \approx 0$.

In assessing the current condition (Fig. 1), eq. 1 was used to infer CD_t for each day (equations provided below). In projecting future conditions, the model first attempts to estimate how much change in Gran ALK is likely to occur under a given deposition scenario on any given day. It then computes the value of all the variables on the right side of eq. 1, such that the right side equals the projected future Gran ALK. $[H^+]$ influences the concentration of all chemical species on the right side of the equation, so for projecting future conditions the appropriate pH is converged upon by iteration (bottom of Fig. 1). The currently observed time series of DOC, the CD_ts estimated from current chemistry, and the projected pH are used to compute changes in A^-, based on a set of organic acid assumptions described below. We assumed that the seasonal patterns and magnitudes of both DOC and CD_t remain constant as deposition changes.

Hemond (1990) described the relationship between charge balance acid-neutralizing capacity (CB ANC) and the ALK measured by Gran titration:

$$(2) \qquad \text{CB ANC} = \text{Gran ALK} + y \times \text{DOC}$$

where CB ANC is base cations minus strong acid anions and *y* is a constant (Hemond (1990) suggested 4.6 μequiv./mg DOC). We assumed that, as deposition changes:

$$(3) \qquad \Delta\text{Gran ALK} = \Delta\text{CB ANC}$$

and that

$$(4) \qquad \Delta\text{Gran ALK} = \Delta\text{CB ANC} = \Delta SO_4^{2-} \times (1 - F) \times -1.$$

We assume that each date's shift in CB ANC and Gran ALK is proportional to that date's change in $[SO_4^{2-}]$ and that the total amount of organic ions for each day ($y \times$ DOC) remains unchanged from its current value (however, the fraction of organic ion in dissociated form changes with pH, as explained below). The *F*-factor represents the fraction of acid deposition that is neutralized in the watershed or streambed. Alhough this widely used *F*-factor approach greatly oversimplifies the various nonlinear and threshold-driven processes believed to operate in watersheds (reviewed in Thornton et al. 1990), it can be used to approximate the steady-state behaviour of more mechanistic models (e.g., Cosby et al. 1994). Past studies generally assume that the *F*-factor varies with nonmarine base cation concentrations (C_B), although the form of this relationship is uncertain. Brakke et al. (1990) proposed that *F* be a sine wave function of current C_B whereas Marmorek et al. (1990) and Henriksen and Brakke (1988) made *F* a linear function of original or current C_B, respectively. Applying these three approaches to the low C_Bs of our three streams (see Table 1) suggests *F*-factors in the range of 0.23–0.45, so $F = 0.3$ is a reasonable estimate of the most likely value. However, to provide thorough sensitivity analyses of model performance, we explored a range of *F* values from 0.3 to 0.7.

The change in daily $[SO_4^{2-}]$ ($\Delta[SO_4^{2-}]_t$) is simply

$$(5) \qquad \Delta[SO_4^{2-}]_t = [SO_4^{2-}]_t \times \text{FCAD} \times (1 - \text{FM})$$

where $[SO_4^{2-}]_t$ is the current concentration, FCAD is the fractional change in acidic deposition under a given deposition scenario (e.g., –0.5 for a 50% decrease in SO_4^{2-} deposition), and FM is the fraction of $[SO_4^{2-}]$ that is from marine sources. We explored the effects of changes in anthropogenic SO_4^{2-} deposition ranging from 100% decreases (i.e., no deposition) to 100% increases. FM was computed based on the correction equations of Vet et al. (1986) and applied to flow-weighted mean stream concentrations of Cl^-, SO_4^{2-}, Ca^{2+}, Na^+, and Mg^{2+}. We estimated C_B using the method of Thompson (1982*a*). Table 1 lists the estimated percent $[SO_4^{2-}]$ from marine sources and the ion ratio used for correction for each of the three streams.

Concentrations of HCO_3^-, CO_3^{2-}, and OH^- are computed using standard equilibrium equations (Stumm and Morgan 1981). In the absence of accurate field measurements of CO_2, we assumed a constant default pCO_2 value of 3.42 indicative of 20% supersaturation ($10^{-3.42} \approx 10^{-3.5} \times 1.2$), consistent with measurements of dissolved O_2. The shallow depth and moderate gradient of these streams probably encourage degassing of CO_2 that enters the stream channel in groundwater and soil water. In sensitivity analyses, we varied $[CO_2]$ over a 10-fold range (i.e., pCO_2 from 2.5 to 3.5), consistent with the range observed in lakes (Norton and Henriksen 1983).

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In TSTM, the amount of dissociated A^- can be specified using monoprotic, diprotic, or triprotic formulations (i.e., one, two, or three K_a values; Lam et al. 1989) or via the formulae of Oliver et al. (1983). As shown by Lam et al. (1989), in all cases, this can be represented by

$$(6) \qquad [A^-] = CD_t \times DOC \times \left[\frac{[K_{a_1} \times x]}{[K_{a_1} \times x + H^+]} \right]$$

where CD_t is the charge density (μequivalents of A^- per milligram of DOC if all A^- are dissociated), x is a scalar derived from the specified pK_as, and the term in square brackets is the fraction of A^- actually dissociated, given the pH. We varied pK_a as a function of pH, following Oliver et al. (1983), because this method was derived from analyses of Nova Scotia waters similar to those in the streams we examined. The CD_t is calculated for each sampling date from current data to precisely match the daily measured current pH and Gran ALK and the estimated HCO_3^-, CO_3^{2-}, and OH^-. This can be done by rearranging eqs. 1 and 6 so that

$$(7) \qquad CD_t = \frac{[\text{Gran ALK} - HCO_3^- - CO_3^{2-} - OH^- + H^+] \times [K_{a_1} \times x + H^+]}{[DOC \times K_{a_1} \times x]}$$

The CD_t computed for each sampling date are applied to all future deposition scenarios, thereby assuming that the hydrological and microbiological processes that determine the seasonal pattern of DOC and CD_t will remain unchanged with changes in deposition. Because eq. 7 can generate negative estimates for CD_t, we "trapped" estimates less than 0.1 and reset them to 0.1. This method of computing CD_t absorbs the effects of any other incorrect assumptions (e.g., incorrect pCO_2 or pK_a values, changes in equilibrium constants due to temperature fluctuations, changes in the protonization of other ions). We therefore compared CD_t with other studies, examined the relationship between CD_t and DOC over a range of pCO_2 values, and explored the effects of a range of pK_a values (pK_a from 3 to 5).

We examined the biological effects of changes in SO_4^{2-} deposition and uncertainties in chemical parameters to help focus attention on the range of values most strongly affecting juvenile salmon production. We chose two output indicators: minimum annual pH and numbers of smolts per square metre assuming an egg deposition of 2.4 eggs/m^2. The version of ASRAM used to compute smolt output was identical to that described in Korman et al. (1994), except that we maintained the ratio of 1-year-old parr to 2-year-old parr at a constant level (11.7:1 for the Westfield River; Lacroix and Korman 1996). As a result, smolt output is slightly more responsive to changes in pH than if the ratio of 2-year-old smolts to 3-year-old smolts varied with parr density as in Korman et al. (1994).

Results

Charge densities

The dynamic CD approach much more accurately tracked pH fluctuations in the Westfield River than did application of a constant CD (Fig. 2). This constant CD was the best possible single value, set to the mean of dynamic CD values. Using a constant CD caused overestimates of pH during low-DOC periods in July, September, and October and pH underestimates in March–May when ALK was low (Fig. 2; and see Fig. 4).

Both pH and DOC affect CD_t. As expected from eq. 7, CD_t varied inversely with DOC, both within and among streams (Fig. 3). The CD_t values are more sensitive to DOC in Moose Pit Brook than in the Westfield River, due to the greater DOC range in Moose Pit Brook (Fig. 3). The effects of pH on CD_t are not intuitively obvious from eq. 7 (since H^+ affects several terms in the numerator), but the net effect is clear from the results. CD_t was positively correlated with pH both over the complete data set (Pearson product moment $r = 0.62$, $P < 0.01$) and also within each of the three streams. CD_t values were more sensitive to variation in pH in the Westfield River than in Moose Pit Brook (Fig. 3). On a seasonal basis (bottom panel of Fig. 4), the assignment of CD_t by the model apparently minimizes the week-to-week fluctuations in the calculated amount of A^- in order to maintain the charge balance in eq. 1. It is likely that pK_a values also change over time (independent of the pH) and that the computed CD_t values absorb some of the seasonal variation in pK_a.

The CD_t values for the Westfield River were more sensitive to variation in assumed pCO_2 than for the other two streams (Table 2), but changes in pCO_2 still had only a minor effect on smolt production (Fig. 5). Increasing CO_2 changes H^+ and HCO_3^-; the net effect is that less A^- (hence, lower CD_t) is required for charge balance (eq. 1). CD_t estimates are less responsive to variation in pCO_2 in the two lower-pH streams, which have both a smaller fraction of total inorganic C as HCO_3^- and more DOC. When we assumed a pCO_2 level of 2.5, we found that in about 20% of the sampling dates the model ended up computing a CD_t that was negative in order to balance eq. 1 (i.e., too much HCO_3^- for the measured Gran ALK), but a pCO_2 of 3.5 yielded no negative CD_t values.

We explored the effect on Westfield River simulations of estimating CD_t using a constant monoprotic representation of organic acids, rather than the Oliver et al. (1983) approach where pK_a varies with pH. We also did this to understand the effects of K_a on CD_t, pH, and smolt production because K_a appears in both the numerator and denominator of eq. 7. As pK_a increased from 3 to 5, CD_t estimates for the Westfield River also increased (from 0.5–2.3 to 1.0–3.4), A^- concentrations increased, and smolt production declined (Fig. 5). Smolt production estimates were relatively insensitive when pK_a varied from 4 to 5 (Fig. 5).

Fraction of marine SO_4^{2-} (FM)

As FM increased, less of the stream SO_4^{2-} (and therefore, ANC) was subject to change through changes in emissions (eq. 5). Consequently, a 30% reduction in deposition at FM = 0.3 caused less of an increase in both pH and smolt production than at FM = 0.1 (Fig. 5). There was very little variation in FM among the three streams simulated here (Table 1), but regional-scale simulations would need to consider this.

Interactive effects of *F*-factor and deposition level

As the *F*-factor is increased, it took a greater reduction in SO_4^{2-} deposition to attain a nontoxic minimum pH (Fig. 6). At $F = 0.3$, a 30% reduction in SO_4^{2-} deposition restored pH levels in the Westfield River to above 5.0 whereas a 70% reduction was required at $F = 0.7$ (Fig. 6). Conversely, higher *F*-factor values conferred a greater resistance to large deposition increases (Fig. 6). We chose a 30% reduction in

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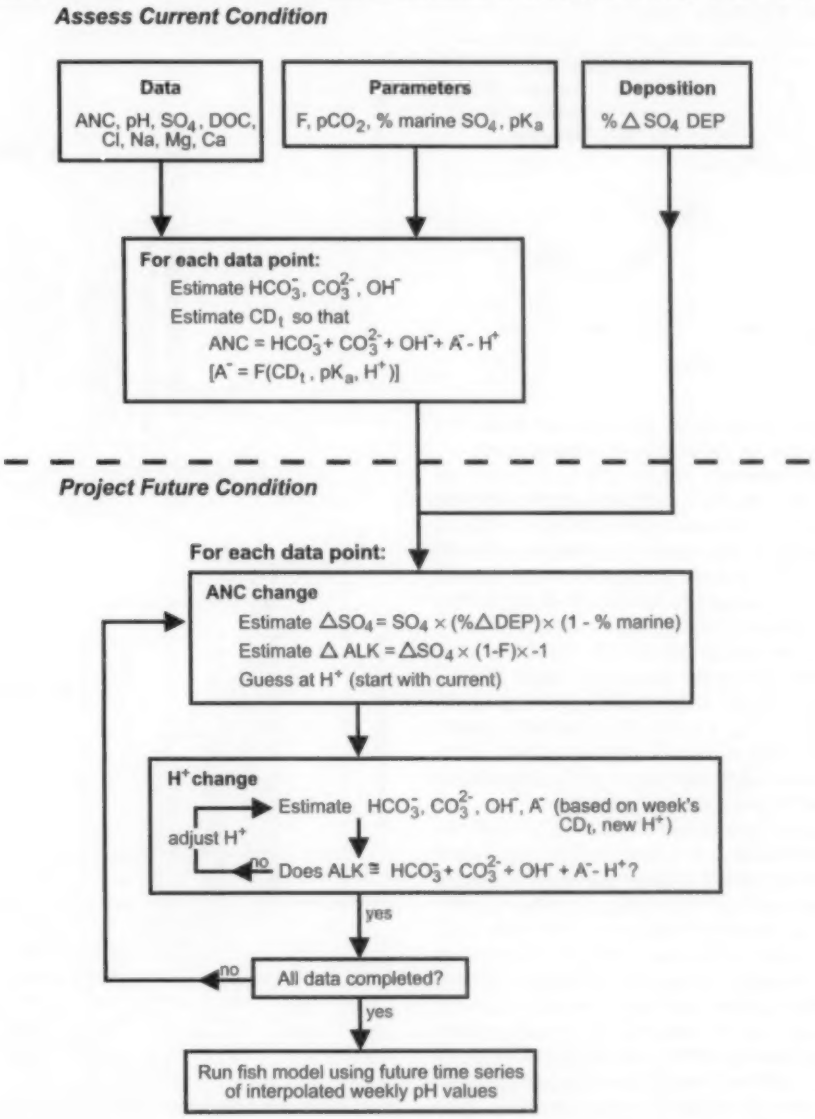
Fig. 1. Flowchart of the time series translation model (TSTM): inputs and structure and procedures for calibration and future projection. FM, fraction of $[SO_4^{2-}]$ from marine sources; FCAD, fractional change in acidic deposition; CD_t, dynamically calculated charge density. Accurate estimates of FM and *F* require stream measurements of Cl^-, SO_4^{2-}, Na^+, Mg^{2+}, and Ca^{2+}.


Assess Current Condition
Data
ANC, pH, SO4, DOC, Cl, Na, Mg, Ca
Parameters
F, pCO2, % marine SO4, pKa
Deposition
% Δ SO4 DEP
For each data point:
Estimate HCO3-, CO3 2-, OH-
Estimate CDt so that
ANC = HCO3- + CO3 2- + OH- + A- - H+
[A- = F(CDt, pKa, H+)]
Project Future Condition
For each data point:
ANC change
Estimate ΔSO4 = SO4 × (%ΔDEP) × (1 - % marine)
Estimate Δ ALK = ΔSO4 × (1-F) × -1
Guess at H+ (start with current)
H+ change
Estimate HCO3-, CO3 2-, OH-, A- (based on week's CDt, new H+)
adjust H+
no
Does ALK ≅ HCO3- + CO3 2- + OH- + A- - H+?
yes
no
All data completed?
yes
Run fish model using future time series of interpolated weekly pH values

deposition for Fig. 5 because it maximized the sensitivity of biological predictions in the Westfield River to variations in *F*. Through the most likely range for Westfield's *F* (0.3–0.4), there was not much change in predicted smolt output (about 0.003 smolt/m^2; Fig. 5). By contrast, changing *F* from 0.6 to 0.7 changed smolt output by two and a half times as much (0.008/m^2; Fig. 5). The zone of greatest parameter sensitivity (i.e., *F* and FCAD) will vary from stream to stream. Also, the *F*-factor may vary seasonally (see Discussion).

We used the model to translate the measured SO_4^{2-}, ALK, and pH into future conditions using two *F*-factors (0.3 and 0.7; Figs. 4 and 6). A 50% reduction in anthropogenic SO_4^{2-} deposition (FCAD = 0.5) resulted in a 40% decrease in $[SO_4^{2-}]$ because marine sources constitute about one fifth of stream $[SO_4^{2-}]$ (Fig. 4). The change in ALK was proportional to but smaller in magnitude than changes in $[SO_4^{2-}]$ due to the watershed neutralization effects represented by the *F*-factor. Changes in pH were more variable over time than changes in $[SO_4^{2-}]$ and ANC because of the nonlinearities inherent in the equilibrium calculations. A large jump in pH occurred during periods when the decrease in $[SO_4^{2-}]$ was sufficient to raise the ALK to the steep part of the ALK–pH relationship (July–November in Fig. 4). With an *F*-factor assumption of 0.3 (the most likely *F*-value), the pH rose to as high as 6.5 in Westfield River

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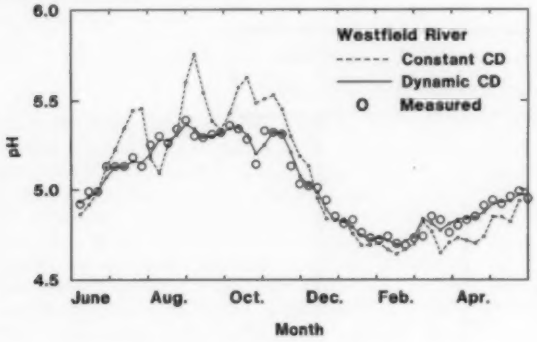
Fig. 2. Comparison of constant and dynamic CD calculations of pH with actual pH measured in the Westfield River during 1983–1984. The constant CD line applied a CD = 1.8, the average of the dynamic CD_t values (Table 2).

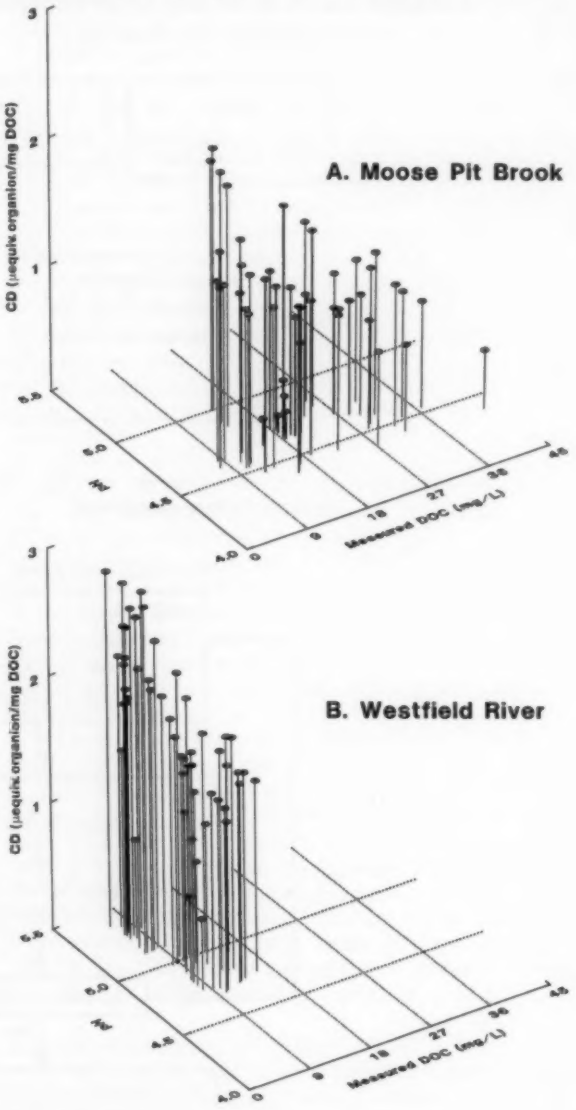


(Fig. 4), but in Moose Pit Brook the pH remained below 6.0 for most of the year due to the effects of organic acids.

The sensitivity of minimum annual pH to *F*-factor assumptions varied with both the deposition scenario and the stream (Fig. 6). The lines represented by different *F*-factors cross at the 0% change in deposition level because low *F*-factor values cause both a greater pH increase with reduced deposition levels and a greater pH decrease with higher deposition levels. The streams with greatest amounts of DOC (Round Lake Brook and Moose Pit Brook) showed a more modest change in the minimum annual pH than did the Westfield River as the result of the buffering effects of organic ions. The results in Fig. 6 imply that even with a complete removal of anthropogenic SO_4^{2-} deposition, Moose Pit Brook would still occasionally experience pH minima below 5 and therefore some Atlantic salmon mortality.

Although the required data inputs to TSTM and ASRAM are modest, the combined models do require frequent chemical measurements to accurately capture the acidic episodes that kill fish. We explored the sensitivity of model predictions to variations in sampling frequency. We first subsampled the weekly data for the three streams to simulate biweekly, triweekly, monthly, or seasonal sampling regimes, then interpolated these coarser data sets to weekly intervals (i.e., the frequency required in ASRAM), and finally ran the "pseudo-weekly" data through TSTM and ASRAM for 0, 25 (for Westfield only), and 50% deposition changes. The results (Fig. 7) illustrate how longer sampling intervals (particularly seasonal) can lead to an overestimate of fish production due to missed acidic episodes or pH minima. Interestingly, this was only a problem for Moose Pit Brook when deposition was reduced by 50% but not under the current highly toxic conditions (Fig. 7). The Westfield River had less variable chemistry than Moose Pit Brook in 1983–1984 and so was less affected by changes in sampling intervals. This analysis suggests that Watt (1987) may have underestimated lost Atlantic salmon production because his classification of streams was based largely on seasonal sampling of stream chemistry. Similarly, the projections in the LaHave River (Korman et al. 1994) were probably biased toward overproduction because the input chemistry data only had a monthly resolution. This overestimation would be

Fig. 3. Calculated CD versus measured DOC and measured pH for (A) Moose Pit Brook and (B) Westfield River.



greatest in the most acidic tributaries, or those where stream chemistry is temporally more variable, where errors would also have the greatest impact on model projections for the river system.

Not surprisingly, streams of intermediate toxicity (i.e., pH 4.7–5.3) require the most frequent sampling to accurately assess current smolt production (D.R. Marmorek, unpublished analyses). Whereas frequent sampling of streams with very low or circumneutral pH may be unnecessary to estimate *current* salmon production levels (i.e., mortality is either 100 or 0%), reliable projections of *future* production do require at least biweekly (preferably weekly) sampling during the fall and winter periods of lowest pH, as altered

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Fig. 4. Current measured levels of pH, ALK, SO_4^{2-}, and DOC, estimated CD_t, new SO_4^{2-} after a 50% decrease in deposition, and projected range of future ALK and pH (F from 0.3 to 0.7) for the Westfield River.

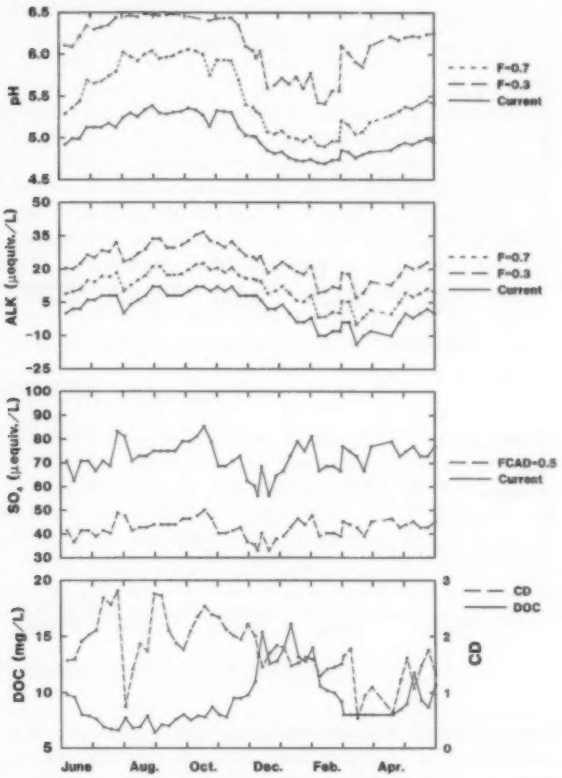


Table 2. Effects of pCO_2 on range and mean value of model-estimated CD_t.

	pCO_2 = 2.5 (lower range)			pCO_2 = 3.5 (upper range)		
Stream	Mean CD_t	Min. CD_t	Max. CD_t	Mean CD_t	Min. CD_t	Max. CD_t
Westfield River	1.1	0.1	1.8	1.8	0.5	2.8
Round Lake Brook	1.2	0.1	1.8	1.5	0.3	2.2
Moose Pit Brook	1	0.1	1	1.1	0.1	2.1

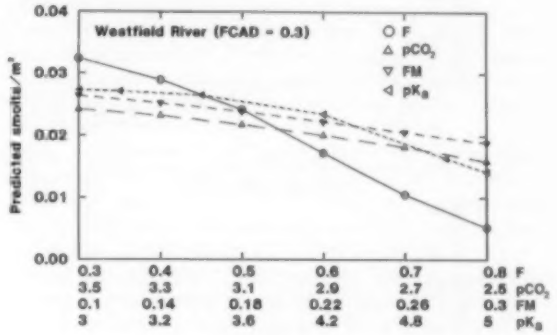
deposition may move the pH of the stream into the zone of intermediate toxicity.

Discussion

Model assumptions and limitations

The key assumptions in TSTM are F-factor values, pCO_2 levels, and CD_ts. The simplifications involved in F-factor models have been discussed in detail in Thornton et al. (1990, chap. 4). We assumed a reasonable F-factor value (0.3) based on the very low C_B values in these streams (61–73 μequiv./L; Table 1). More accurate estimates of F require

Fig. 5. Effects of independently varying F, pCO_2, FM, and pK_a over a wide range of possible values on predicted Atlantic salmon smolt output in the Westfield River (1983–1984) with a 30% decline in SO_4^{2-} deposition. Default values used: F = 0.5, pCO_2 = 3.42, FM = 0.174, pK_a using Oliver et al. (1983). Parameter sensitivity varied with the stream and deposition scenario.



a period with changing SO_4^{2-} deposition, which has not occurred in this region. The greatest risk of the F-factor approach is underestimating the damage from acid deposition by not accounting for soil reservoirs of C_B and SO_4^{2-} (Labieniec et al. 1989; Cosby et al. 1994). Seasonal variations in F due to changes in hydrology and geochemical processes are not explicitly represented in TSTM, but are implicit in the current patterns of stream chemistry, which in turn affect how TSTM distributes the impacts of deposition changes over the year.

We assumed a constant pCO_2 level (3.42). Several other models vary pCO_2 seasonally with temperature (e.g., Cosby et al. 1985) or assign a lower pCO_2 value, indicating considerable CO_2 supersaturation (e.g., Thompson 1982*b*). However, the small streams we modelled are extremely well mixed, and assuming only a small amount of supersaturation seems reasonable. Only the Westfield River showed significant chemical or biological sensitivity to changes in assumed pCO_2 (Table 2; Fig. 5). This is because its higher pH allows for a greater fraction of carbonic acid to be present as HCO_3^- and because its pH levels straddle the toxic zone.

Previous modelling efforts have generally assumed a constant CD (reviewed by Thornton et al. 1990). There is, however, some empirical justification for assuming that CD varies seasonally with changes in the ratio of humic (lower CD) to fulvic (higher CD) acids (Visser 1984). Our estimates of CD_t decreased from August to March in the Westfield River (Fig. 4), although we do not have empirical evidence that CD_t actually varied in this manner. Obtaining more accurate estimates of CD_t for these streams (e.g., as in Driscoll et al. 1994) would help to test our assumed structure.

Are our estimates of CD_t reasonable? CD_ts calculated by TSTM (1–3 μequiv./mg DOC; Fig. 3) were about 3–4 μequiv./mg lower than those in three other studies (Eshelman and Hemond 1985; Gorham et al. 1985; Wilkinson et al. 1992) but close to the estimates of Driscoll and Schafran (1984) and Driscoll et al. (1994) for the

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Fig. 6. Variation in projected minimum annual pH with changes in deposition (± 100%) and assumed *F*-factor (0.3, 0.5, 0.7) for the three modelled streams.

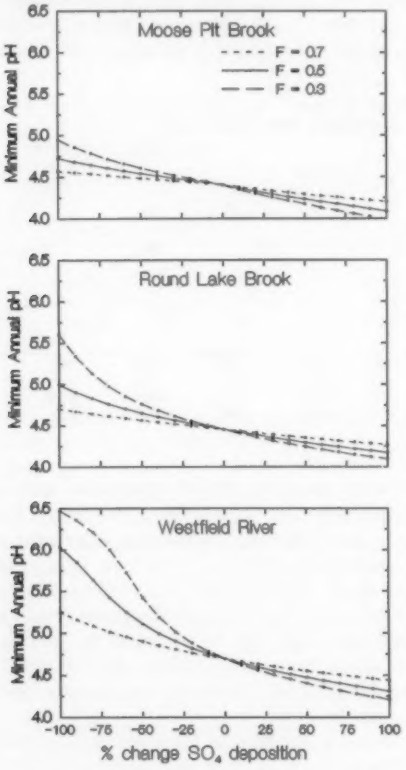
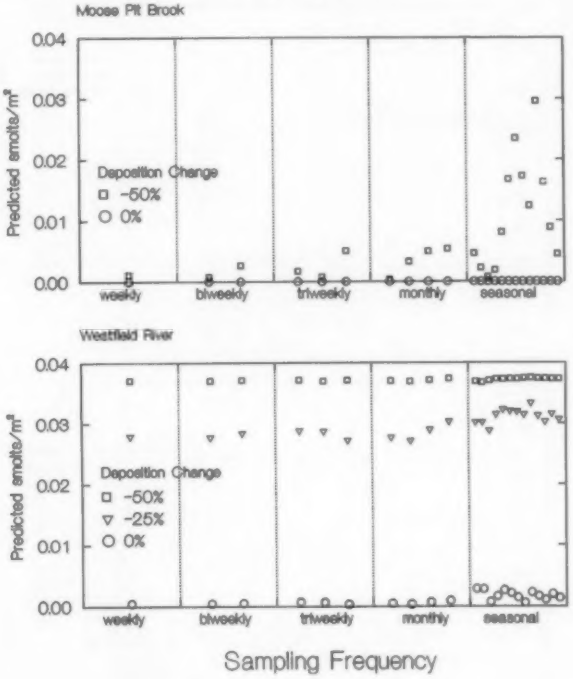


Fig. 7. Simulated effect of sampling frequency on predicted Atlantic salmon smolt output for Moose Pit Brook and Westfield River under 0% (circles), –25% (Westfield only (triangles)), and –50% (squares) changes in SO_4^{2-} deposition.



Adirondacks (2.1 and 2.5–4.2). (Values were calculated from fig. 1*b* of Driscoll et al. (1994) for data with pH <5.3 assuming 1 equiv./mol DOC = 83.33 μequiv./mg DOC.) Adirondack surface waters are similar to Nova Scotia streams in two other respects: both show a positive correlation between CD_t and pH (Driscoll et al. 1994; our Fig. 3) and organic acids play a significant role in later summer and fall acidic episodes in both (Lacroix and Korman 1996; Wigington et al. 1996). Our CD_t values declined with DOC (Fig. 3), a pattern consistent with the results of Wilkinson et al. (1992). We believe that the primary reason that our CD_t estimates are lower than some other studies is that the streams we examined had lower pH values and higher DOC concentrations, both of which act to decrease CD_t.

In TSTM, we assumed that CD_t and DOC remained unchanged for each sampling date as we changed the amount of acidic deposition. There is evidence from a variety of sources that DOC can change with acidic deposition or drought (e.g., Yan et al. 1996), although the magnitude of change is uncertain (Marmorek and Bernard 1990). The critical issue here is whether the total amount of A^- changes. If the generally observed inverse relationship between DOC and CD_t is maintained (Fig. 3), then even if DOC were to change with changes in acidic deposition, the shift in total amounts of A^- (product of DOC and CD) may not be large enough to have a significant effect on stream pH. Clair and Ehrman (1995) detected declining total organic C (TOC) trends in 15 of 18 Nova Scotia streams between 1983 and 1992, which they attributed to reduced runoff and watershed export of TOC and proportionately greater fractions of low-DOC groundwater in stream flow. Although annual mean DOC levels were lower in our streams during this period, the autumn DOC peaks and associated pH minima were more extreme than in years with typical precipitation levels (G.L. Lacroix, unpublished data). Very dry summers followed by typically wet falls may be creating an extra acidification effect in Nova Scotia streams similar to that observed by Yan et al. (1996) after a 2-year drought in northern Ontario.

TSTM provides a picture of the chemical conditions expected under different levels of SO_4^{2-} deposition. It reproduced the seasonal pattern of pH fluctuations currently observed and translated these patterns in a reasonable way. TSTM has several limitations: it does not provide any estimate of how long it might take a watershed to reach a steady-state condition associated with a given level of acid deposition, it does not consider N deposition or long-term changes in climate, and it should not be applied to clear streams with low DOC where Al^{n+} is much more important. The advantage of TSTM, however, is that it does permit an exploration of chemical responses on fine time scales in a realistic way with a minimum amount of data inputs and, when linked to ASRAM, generates projections for Atlantic salmon production as well.

Regional projections of impacts using TSTM–ASRAM

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will require estimates of a range of potential smolt production values from well-monitored index streams representing different pH–DOC classes. We recommend pH cruises (i.e., detailed snapshots of spatial pH distributions) of other tributaries during the fall and winter to classify the percentage of salmon production areas within these pH–DOC classes and to estimate C_Bs for *F*-factor estimates. Then the smolt production ranges derived from TSTM–ASRAM runs can be applied to generate projections of salmon production within different river basins under different deposition scenarios. The critical assumption in this procedure is that the subset of streams sampled and simulated must be representative of the larger population of streams in southwest Nova Scotia. As improvements are made in the spatial and temporal resolution of chemical data available for acidified salmon rivers in southwest Nova Scotia, we should be able to evaluate the regional impacts of various deposition scenarios and the potential for recovery using the proposed TSTM–ASRAM.

Acknowledgements

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Comparison of methods to account for autocorrelation in correlation analyses of fish data

Brian J. Pyper and Randall M. Peterman

Abstract: Autocorrelation in fish recruitment and environmental data can complicate statistical inference in correlation analyses. To address this problem, researchers often either adjust hypothesis testing procedures (e.g., adjust degrees of freedom) to account for autocorrelation or remove the autocorrelation using prewhitening or first-differencing before analysis. However, the effectiveness of methods that adjust hypothesis testing procedures has not yet been fully explored quantitatively. We therefore compared several adjustment methods via Monte Carlo simulation and found that a modified version of these methods kept Type I error rates near α. In contrast, methods that remove autocorrelation control Type I error rates well but may in some circumstances increase Type II error rates (probability of failing to detect some environmental effect) and hence reduce statistical power, in comparison with adjusting the test procedure. Specifically, our Monte Carlo simulations show that prewhitening and especially first-differencing decrease power in the common situations where low-frequency (slowly changing) processes are important sources of covariation in fish recruitment or in environmental variables. Conversely, removing autocorrelation can increase power when low-frequency processes account for only some of the covariation. We therefore recommend that researchers carefully consider the importance of different time scales of variability when analyzing autocorrelated data.

Résumé : L'autocorrélation du recrutement des poissons et des données écologiques peut compliquer l'inférence statistique dans les analyses de corrélation. Pour régler ce problème, les chercheurs ajustent souvent les méthodes de vérification des hypothèses (p. ex. ajustement des degrés de liberté) afin de tenir compte de l'autocorrélation, ou éliminent l'autocorrélation par l'introduction de bruit blanc ou le calcul des différences premières. Toutefois, l'efficacité des méthodes servant à l'ajustement des méthodes de vérification des hypothèses n'a pas encore été entièrement étudiée sur le plan quantitatif. Nous avons donc comparé plusieurs méthodes d'ajustement par la simulation de Monte Carlo et nous avons constaté qu'une version modifiée de ces méthodes permet de maintenir le taux d'erreur de type I près de α. Par contraste, les méthodes qui éliminent l'autocorrélation contrôlent bien le taux d'erreur de type I mais dans certains cas, elles augmentent le taux d'erreur de type II (probabilité de ne pas déceler certains effets écologiques) et donc, réduisent l'efficacité statistique comparativement à l'ajustement de la méthode de vérification. Plus particulièrement, nos simulations de Monte Carlo montrent que l'introduction de bruit blanc, et surtout le calcul des différences premières, diminuent l'efficacité dans des situations ordinaires où les processus à basse fréquence (à évolution lente) sont des sources importantes de covariation du recrutement des poissons ou des variables de l'environnement. Réciproquement, l'élimination de l'autocorrélation peut accroître l'efficacité statistique lorsque les processus à basse fréquence ne représentent qu'une certaine partie de la covariation. Nous recommandons donc aux chercheurs de considérer attentivement l'importance des différentes échelles de temps de la variabilité lorsqu'ils analysent des données autocorrélées.

[Traduit par la Rédaction]

Introduction

One of the greatest challenges in fish biology and management is to understand mechanisms associated with highly variable survival rates and recruitment in fish populations. This variability can occur over various time scales or frequencies. For instance, rapid year-to-year changes (variability at higher frequencies) in fish survival rates, zooplankton abundance, and oceanographic variables are commonly observed characteristics of marine ecosystems, where values of a given variable are largely independent from one year to the next. In recent years, however, fisheries scientists and oceanographers have found increasing evidence of slowly changing, long-term variations (variability at lower frequencies) in these biological and physical variables (e.g., Beamish 1995), such that values in a given year are closely related to values in previous years (i.e., the data contain positive autocorrelation). This longer term variability has been exhibited in the Northeast Pacific Ocean, for example, by extended periods of unusually high sea-surface temperatures, weak upwelling, and low biological productivity followed by periods of the opposite.

Correlation analysis has been a useful and widely applied tool for generating hypotheses about the effects of environ-

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mental or other variables on recruitment at these various time scales (e.g., Myers et al. 1995*a*, and papers cited therein). However, a major statistical challenge exists when time series of recruitment and environmental data are strongly autocorrelated, i.e., dominated by low-frequency variability (e.g., Chelton 1984; Thompson and Page 1989). Such autocorrelation violates the assumption of serial independence required for most classical inference tests (Hurlbert 1984). In general, this means that a sample correlation between two autocorrelated time series has fewer degrees of freedom (or a larger variance) than that assumed under the classical significance test. Consequently, the test will have a Type I error rate greater than the specified α (i.e., there will be an increased chance of concluding that a correlation is statistically significant when in fact no correlation is present; Jenkins and Watts 1968, p. 338).

To address this problem, fisheries scientists and oceanographers have typically applied two qualitatively different approaches. One approach is to modify the hypothesis testing procedure by computing either a corrected degrees of freedom for the sample correlation (Garrett and Petrie 1981; Chelton 1984) or, equivalently, a corrected variance for the sample correlation (Kope and Botsford 1990). The usefulness of these methods has been questioned, however, because they depend on the autocorrelation function of each time series, which can be poorly estimated for short time series typical of fisheries data sets (Thompson and Page 1989). Despite the importance of this statistical problem, we are not aware of any publications examining the ability of these methods to control Type I error rates. This paper therefore attempts to fill this gap.

The second approach to dealing with autocorrelation attempts to remove it from each time series of data before computing and testing correlations. This has been done either by fitting time series models to the data and using residuals from them (often referred to as "prewhitening"; e.g., Milicich et al. 1992; Quinn and Niebauer 1995) or by "first-differencing" the data (subtracting each data point from the next; e.g., Thompson and Page 1989). The premise behind these methods is that if the new transformed data series are free of autocorrelation, then classical inference tests are appropriate for correlations computed between them. However, because removing autocorrelation is equivalent to removing low-frequency variability from data, a potential drawback of prewhitening and first-differencing seems clear: if the low-frequency components of variability in two time series of recruitment or environmental data are *common* (i.e., synchronous or asynchronous), as some researchers are finding (see Beamish 1995), then removing autocorrelation may also remove much of this covariance (Thompson and Page 1989). Such procedures may thus increase the probability that researchers will miss finding some important relationship between long-term, slowly changing environmental processes and fish population dynamics. In other words, they may face an increased Type II error rate (i.e., probability of failing to reject the null hypothesis of no correlation when it is in fact false). In many of the papers we reviewed, however, the researchers did not seem to recognize this potential drawback of removing autocorrelation.

This paper has two main objectives. First, we use Monte Carlo simulations to examine the effectiveness of several commonly employed methods for adjusting the test procedure of the null hypothesis of no correlation between autocorrelated data (Garrett and Petrie 1981; Chelton 1984; Kope and Botsford 1990). Specifically, we examine how closely the Type I error rates of these methods match the specified α. Second, we use a simple model and Monte Carlo simulations to illustrate the degree to which prewhitening and first-differencing can alter levels of real covariation between autocorrelated data and affect Type II error rates in comparison with the best method for adjusting the test procedure. For completeness, we also examine how levels of covariation and Type II error can be altered by "smoothing" data, a method that, in contrast with prewhitening and first-differencing, is frequently used to remove high-frequency components of variability from data. In addition, we provide an empirical example using recruitment data for several stocks of sockeye salmon (*Oncorhynchus nerka*) from Bristol Bay, Alaska. We limit our analyses of Type I and Type II error rates to the case where correlations are being computed at a single lag, which is typical of most fisheries applications. Furthermore, because our main intended audience is fisheries biologists, all methods and results are in the context of the time domain (i.e., correlations and autocorrelations), rather than the frequency domain often used by oceanographers (i.e., spectral analysis).

Adjusting the test procedure of a sample correlation

Garrett and Petrie (1981), Chelton (1984), and Kope and Botsford (1990) provide similar methods for adjusting the null hypothesis test of a sample correlation between two autocorrelated time series, say X and Y. These methods, which are based on formulas from either Bayley and Hammersley (1946) or Bartlett (1946), assume that the time series are stationary, such that their underlying means remain constant over time. Furthermore, when testing a sample correlation at a given lag k, denoted $r_{XY}(k)$, each of these methods assumes a null hypothesis of no correlation at *all* lags.

These methods can be summarized using the following theoretical approximation of the "effective" number of degrees of freedom, N^*, of $r_{XY}(k)$

$$(1) \qquad \frac{1}{N^*} \approx \frac{1}{N} + \frac{2}{N}\sum_{j=1}^{\infty}\frac{(N-j)}{N}\rho_{XX}(j)\rho_{YY}(j)$$

where N is the sample size and $\rho_{XX}(j)$ and $\rho_{YY}(j)$ are the autocorrelations of X and Y at lag j. For example, Garrett and Petrie (1981) used a form of eq. 1 where $\rho_{XX}(j)\rho_{YY}(j)$ was replaced by $\rho(j)$, the autocorrelation at lag j of the *cross-product* of X and Y (each standardized to have a mean of zero). Given N^*, Garrett and Petrie (1981) used the standard critical value for $r_{XY}(k)$ at the α significance level that can be read from statistical tables or derived using the t distribution for either one- or two-tailed tests (Zar 1984, p. 309):

$$(2) \qquad r_{crit} = \sqrt{t^2_{\alpha,N^*}/(t^2_{\alpha,N^*} + N^*)}.$$

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Chelton (1984) recommended an expression for N^* that is equivalent to eq. 1 but without the weighting function $(N - j)/N$. Chelton also recommended an alternative critical value based on the chi-square distribution with 1 degree of freedom, which is equivalent to the following, based on the standard normal distribution (Z):

$$(3) \qquad r_{crit} = Z_{\alpha/2}\sqrt{1/N^*}$$

where values of α and $\alpha/2$ are used for one- and two-tailed tests, respectively.

Kope and Botsford (1990) provided an approximation for the *variance* of a sample correlation that is equivalent to $1/N^*$ as defined by eq. 1. They assumed that the null distribution of $r_{XY}(k)$ is normal, which also implies the critical value defined by eq. 3. Thus, the only difference between their method and that of Chelton is that Kope and Botsford included the weighting function $(N - j)/N$ in the computation of N^* (eq. 1).

Each of the above methods for adjusting the test procedure attempts to maintain Type I error rates at the specified α by generating critical values that account for autocorrelation. When both time series contain positive autocorrelation, it is clear from eq. 1 that values of N^* will be less than N, resulting in larger, and hence more conservative, critical values (i.e., fewer rejections of the null hypothesis of no correlation than if no adjustment were made). However, these methods may perform poorly in practice for a number of reasons: (*i*) the expressions for N^* are based on asymptotic (large sample size) formulas that may not be accurate for short time series, (*ii*) values of N^* depend on estimates of autocorrelation functions that are known to be both imprecise and biased, especially for short time series (Jenkins and Watts 1968, chap. 5), and (*iii*) the distributions assumed by critical values (2) and (3) may be inappropriate, particularly for short time series with high levels of autocorrelation. In addition, it is unclear how many lags j should be used when computing N^*. Given these potential problems, we conducted the following simulation analysis to examine the performance of these methods.

Methods—adjusting the test procedure

We used simple time series models to generate hypothetical data sets with lengths (number of years) and levels of positive autocorrelation that were typical of recruitment and environmental time series. Recruitment series used in correlation analyses often vary from about 15 years to usually not more than 50 years (e.g., Myers et al. 1995*a*), and it is common to observe estimates of lag-1 autocorrelation ranging from moderate (e.g., 0.5) to high values (e.g., 0.8) (e.g., Koslow et al. 1987).

In our baseline analysis, we simulated two *independent* time series X and Y of length N using first-order autoregressive models (AR(1) models) (Box and Jenkins 1976, p. 56):

$$(4) \qquad X_t = \phi_X X_{t-1} + \varepsilon_t$$

and similarly for Y, where ε was randomly and normally distributed and ϕ_X is the autoregressive parameter. The variance of X as defined by eq. 4 is $\sigma_X^2 = \sigma_\varepsilon^2/(1 - \phi_X^2)$, which was used to generate the initial value of X. Note that X has a theoretical autocorrelation function

$$(5) \qquad \rho_{XX}(j) = \phi_X^{|j|}$$

such that greater absolute values of ϕ_X give rise to more autocorrelated time series. Also note that the value of ϕ_X corresponds to the theoretical lag-1 autocorrelation in the time series. AR(1) models can produce a wide range of autocorrelated time series and often provide a reasonable description of fisheries and ecological data.

We generated X and Y with N ranging from 15 to 50 years (in increments of 5) and with the same autoregressive parameter ϕ (i.e., $\phi_X = \phi_Y$) ranging from 0.4 to 0.9 (in increments of 0.1). For each combination of N and ϕ, 5000 Monte Carlo trials were done in which we computed sample correlations between the two time series at lag zero (i.e., using the N pairs of simulated data) and tabulated Type I error rates for two-tailed tests of the null hypothesis of no correlation for three levels of α (0.02, 0.05, and 0.10) using the following testing methods: (A) the standard significance test using critical value (2) and $N - 2$ degrees of freedom (i.e., not making any adjustments for autocorrelation), (B) the Garrett–Petrie method, (C) the Chelton method, and (D) the Kope–Botsford method. For methods B–D, autocorrelations were estimated over the first $N/4$ lags (i.e., $j = 1$ to $N/4$ in eq. 1) using an estimator recommended by Box and Jenkins (1976, p. 32):

$$(6) \qquad r_{XX}(j) = \frac{\sum_{t=1}^{N-j}(X_t - \bar{X})(X_{t+j} - \bar{X})}{\sum_{t=1}^{N}(X_t - \bar{X})^2}.$$

On occasion, variability in autocorrelation estimates resulted in values of N^* that were greater than the sample size N. We followed Kope and Botsford (1990) and constrained N^* to a maximum of N.

We examined the sensitivity of the methods to the choice of critical value, autocorrelation estimator, the number of lags for which autocorrelations were estimated, and the type of time series model used in the simulations.

Results and discussion—adjusting the test procedure

A summary of Type I error rates is shown in Table 1 for each of the methods A–D described above. Results for the standard significance test provide a baseline for comparison (Table 1, Case A). In this case, error rates were consistently much greater than the specified α values and increased rapidly as ϕ increased, as illustrated in Fig. 1A for $\alpha = 0.05$. This bias emphasizes the importance of accounting for autocorrelation. However, the efficacy of the methods designed to control Type I error rates varied considerably. The Garrett–Petrie method performed poorly at the three α values (Case B), with error rates larger than α and increasing with ϕ (e.g., Fig. 1B). In contrast, the Chelton and Kope–Botsford methods gave very similar error rates (Cases C and D) that were somewhat conservative at $\alpha = 0.02$, reasonably accurate although variable at $\alpha = 0.05$, but greater and more variable than desired at $\alpha = 0.10$. Error rates of these two

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Table 1. Summary of simulated Type I error rates for different methods of adjusting the hypothesis test procedure, different time series models, and three levels of α.

		Hypothesis test procedure		Type I error rates					
				α = 0.02		α = 0.05		α = 0.10	
Case	Time series model	Method	No. of lags *j*	Average	Range	Average	Range	Average	Range
A	AR(1)[a]	Standard significance test		0.143	0.034–0.389	0.212	0.080–0.474	0.29	0.15–0.55
B	AR(1)[a]	Garrett–Petrie	*N*/4	0.095	0.027–0.215	0.154	0.063–0.299	0.23	0.12–0.39
C	AR(1)[a]	Chelton	*N*/4	0.013	0.007–0.017	0.056	0.041–0.078	0.14	0.10–0.23
D	AR(1)[a]	Kope–Botsford	*N*/4	0.015	0.008–0.018	0.062	0.043–0.098	0.15	0.10–0.25
E	AR(1)[a]	Chelton using theoretical *N**		0.006	0.000–0.016	0.024	0.000–0.047	0.07	0.00–0.10
F	AR(1)[a]	Chelton; theoretical *N**; critical value (2)		0.013	0.000–0.023	0.037	0.001–0.058	0.08	0.01–0.12
G	AR(1)[a]	Chelton using critical value (2)	*N*/4	0.031	0.019–0.066	0.081	0.051–0.168	0.16	0.11–0.28
H	AR(1)[a]	Modified Chelton	*N*/4	0.018	0.013–0.022	0.053	0.043–0.069	0.12	0.09–0.16
J	AR(1)[a]	Modified Chelton with eq. 7	*N*/4	0.014	0.011–0.017	0.044	0.037–0.047	0.10	0.09–0.12
K	AR(1)[a]	Modified Chelton with eq. 7	*N*/3	0.012	0.009–0.015	0.038	0.032–0.044	0.09	0.08–0.10
L	AR(1)[a]	Modified Chelton with eq. 7	*N*/5	0.016	0.011–0.018	0.047	0.043–0.051	0.11	0.09–0.12
M	AR(1)[a]	Modified Chelton with eq. 7	*N*/6	0.017	0.012–0.021	0.049	0.044–0.057	0.11	0.09–0.14
N	AR(2)[b]	Standard significance test		0.141	0.026–0.384	0.212	0.064–0.469	0.29	0.13–0.55
P	AR(2)[b]	Modified Chelton with eq. 7	*N*/5	0.016	0.011–0.021	0.049	0.040–0.058	0.11	0.09–0.14
Q	AR(2)[c]	Standard significance test		0.118	0.078–0.358	0.265	0.140–0.436	0.35	0.22–0.52
R	AR(2)[c]	Modified Chelton with eq. 7	*N*/5	0.011	0.005–0.021	0.035	0.021–0.061	0.08	0.06–0.13
S	ARIMA(1,1,0)[d]	Standard significance test		0.593	0.303–0.768	0.654	0.395–0.808	0.71	0.48–0.84
T	ARIMA(1,1,0)[d]	Modified Chelton with eq. 7	*N*/5	0.016	0.011–0.021	0.055	0.050–0.062	0.14	0.13–0.15
U	ARIMA(1,1,0)[e]	Modified Chelton with eq. 7	*N*/5	0.047	0.033–0.063	0.114	0.081–0.155	0.22	0.17–0.28

Note: The average and range of each set of simulations were computed across all combinations of *N* (ranging from 15 to 50 in increments of 5) and the parameter values of the time series model. See text for details.

[a]Parameter range for ϕ: 0.4 to 0.9 (increments of 0.1).

[b]Parameter range for ϕ_1: 0.2 to 0.7 (increments of 0.1); ϕ_2: 0.2 to $(0.9 - \phi_1)$ (increments of 0.1).

[c]Parameter range for ϕ_1: 0.8 to 1.8 (increments of 0.2); ϕ_2: $-\phi_1/2$.

[d]Parameter range for ϕ: 0.0 to 0.6 (increments of 0.3).

[e]Parameter value for ϕ: 0.9.

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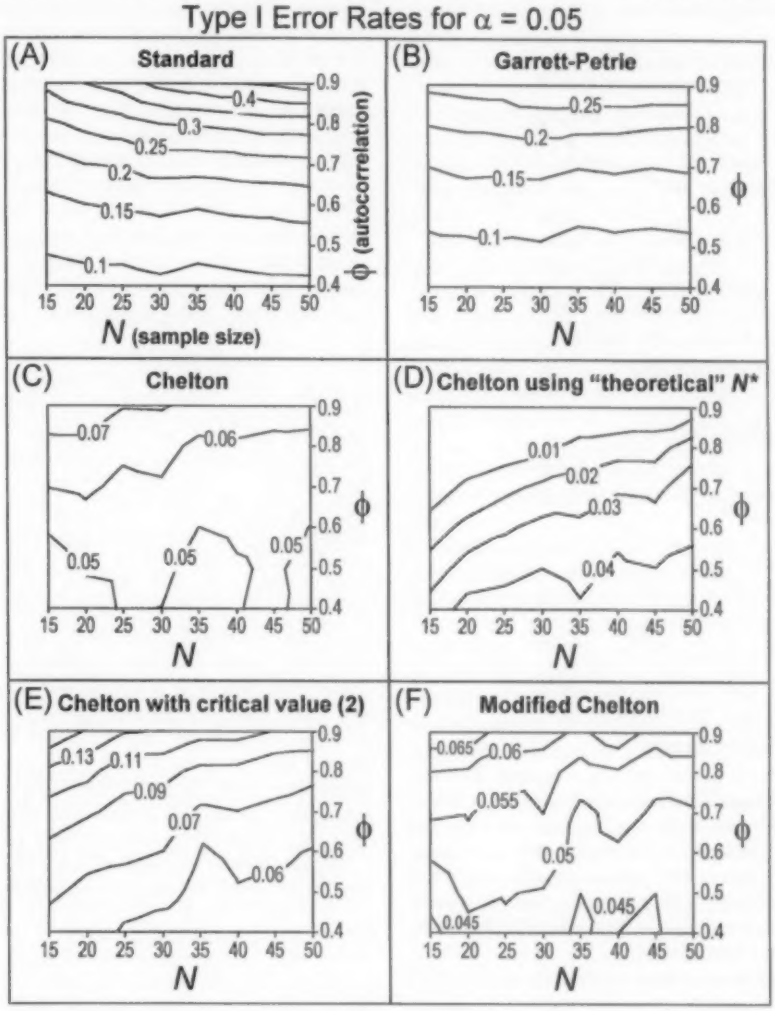
Fig. 1. Isopleths of simulated Type I error rates at $\alpha = 0.05$ for (A) the standard inference test, (B) the Garrett–Petrie method, (C) the Chelton method, (D) the Chelton method using theoretical values of N^*, (E) the Chelton method using critical value (2) instead of critical value (3), and (F) the Modified Chelton method (i.e., using critical value (2) with $N^* - 2$). See text for details.



methods also increased as ϕ increased, especially for small N (e.g., Fig. 1C).

The Garrett–Petrie method was not as effective as the Chelton and Kope–Botsford methods primarily because estimates of $\rho(j)$, the autocorrelation function of the cross-product used in eq. 1, were highly imprecise and often negative for lags greater than 1 or 2, resulting in large overestimates of N^*. Changing the number of lags j used to estimate N^* did little to improve performance. For long time series (e.g., $N = 1000$), we found that estimates of $\rho(j)$ were very similar to those of $\rho_{XX}(j)\rho_{YY}(j)$ (the product used in the Chelton and Kope–Botsford methods), but for short time series, $\rho(j)$ was consistently smaller and much more variable. We therefore recommend that researchers not use the Garrett–Petrie approach.

For a given α value, Type I error rates of the Kope–Botsford method (Table 1, Case D) were slightly larger and more variable than those of the Chelton method (Case C). Recall that the only difference between these methods is that the Kope–Botsford method includes the weighting function $(N - j)/N$ in eq. 1. This added weighting function produced slightly larger estimates of N^*, which, over the various simulations we performed, consistently resulted in error rates that were slightly more variable than those of the Chelton method. We therefore limit further discussions of results to the Chelton method.

We found that the Type I error rates of the Chelton method were largely determined by an interaction among three main factors. First, it appears that eq. 1 (with or without the weighting function) does not provide accurate "theoretical" values of N^* for short, autocorrelated time series. For example, we used the theoretical autocorrelation func-

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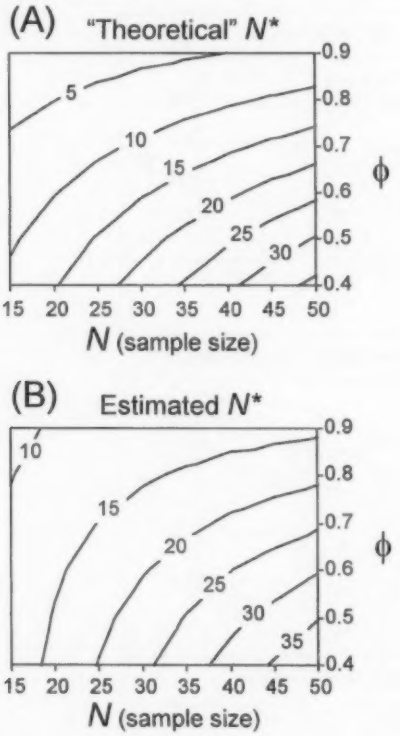
Fig. 2. Isopleths of (A) theoretical values of N^* computed using the theoretical autocorrelation functions of X and Y (eq. 5) and Chelton's form of eq. 1 and (B) average simulated estimates of N^* computed using Chelton's form of eq. 1.



tions of X and Y (eq. 5) and Chelton's form of eq. 1 to compute the theoretical N^* for each combination of N and ϕ (Fig. 2A). However, when these values of N^* were used with critical value (3), error rates were typically much too conservative (Table 1, Case E), falling well below the specified α values as ϕ increased (e.g., Fig. 1D). Results were similar for critical value (2) (Case F).

Second, estimates of autocorrelation were biased low in our simulations (i.e., toward less positive values), resulting in estimates of N^* that were considerably larger than the theoretical values of N^* (Fig. 2). Changing the number of lags j used to estimate N^* did little to reduce these differences. Standard estimators of autocorrelation can be seriously biased in this direction, with biases increasing for shorter time series or larger autocorrelations (e.g., Marriott and Pope 1954). Consequently, these biases compensated somewhat for the fact that theoretical values of N^* are overly conservative at high ϕ (e.g., Fig. 1D), resulting in error rates for the Chelton method that were reasonably accurate for $\alpha = 0.05$ (Fig. 1C).

Third, critical value (3) (based on the normal assumption) is inappropriate for autocorrelated data when N is small, just as it would be for the standard case where data are serially independent and critical value (2) is appropriate (Zar 1984, p. 309). When N is small (e.g., <100), the null distribution of a sample correlation has less weight in its tails (platykurtic) than assumed by critical value (3). This in part explains the tendency for error rates of the Chelton method to be lower than desired at $\alpha = 0.02$ and greater at $\alpha = 0.10$ (Table 1, Case C). Note, however, that using Chelton's form of eq. 1 with the standard critical value (2) gave larger and more variable Type I error rates for all combinations of N, ϕ, and α (Case G), with error rates increasing with ϕ (e.g., Fig. 1E). This result is not surprising given that critical value (3) will be larger than, and hence more conservative than, critical value (2) for a given estimate of N^*. Nevertheless, as we show below, critical value (2) *will* provide better coverage than critical value (3) when appropriate values of N^* are used. Thus, it would seem that the Chelton method (or the similar Kope–Botsford method) performed as well as it did because of a somewhat fortuitous and complex interaction between conservative values of theoretical N^*, biases in autocorrelation, and a conservative critical value (3).

Improvements to the Chelton method

Given these findings, we explored various adaptations of the Chelton method in an effort to improve its performance. The first and most useful adjustment we found was to use the standard critical value (2) instead of critical value (3), but with $N^* - 2$ rather than N^* degrees of freedom. We refer to this as the Modified Chelton method. Across all combinations of N and ϕ, this approach provided a better balance between estimates of N^* and Type I error rates at the three α levels (Table 1, Case H; Fig. 1F). In general, using $N^* - 1$ or $N^* - 3$ resulted in more variable error rates that were too large and too small, respectively. Despite the use of critical value (2), there was still a tendency for error rates to be lower than desired at $\alpha = 0.02$ and greater at $\alpha = 0.10$ (Case H). While this may indicate that critical value (2) is not entirely appropriate for autocorrelated data, it appeared to be largely caused by the fact that N^* is a random variable and hence, so is the critical value. This resulted in something analogous to the null distribution having less weight in its tails than assumed by critical value (2).

Performance was improved even further by using a different autocorrelation estimator (Chatfield 1989, p. 50):

$$(7) \qquad r_{XX}(j) = \frac{N}{N-j} \times \text{eq. 6}$$

This estimator is often preferred to eq. 6 because it is less biased, although more variable (Chatfield 1989, p. 50). Using eq. 7 produced slightly smaller estimates of N^* (by roughly 0.5 on average) that were particularly influential at small N. As a result, error rates were reduced and, more importantly, were consistently less variable (Table 1, Case J). Unless stated otherwise, all further results are reported for the Modified Chelton method using eq. 7 to estimate autocorrelations.

A final factor influencing Type I error rates was the number of lags j for which autocorrelations were estimated in the computation of N^* (eq. 1). For example, increasing this number from $N/4$ to $N/3$ resulted in slightly smaller estimates of N^* (by roughly 0.3 on average) and overly conservative error rates for $\alpha = 0.02$ and 0.05 (Table 1, Case K). In contrast, using $N/5$ gave larger estimates of N^* (by only 0.2 on average) and provided perhaps the best combination of accuracy and precision in error rates for the three α values (Case L). Further reducing the number of lags to $N/6$ gave

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slightly larger and more variable error rates (Case M). Ideally, the optimal number of lags to use in a given situation would depend on both N and the theoretical autocorrelation functions of the time series. For instance, for a given N, error rates increased with ϕ (e.g., Fig. 1F), suggesting that more lags should be used at high ϕ than for low ϕ. However, because the theoretical autocorrelation functions are unknown and estimates of them can be highly variable, a reasonable approach is to use a simple rule, such as $N/5$, that yields reasonably accurate error rates with minimal variability across sample sizes and time series models.

Sensitivity analysis of the Modified Chelton method to various time series models

The results reported above were for the set of AR(1) models where both X and Y were simulated using the same value for the autoregressive parameter ϕ (i.e., $\phi_X = \phi_Y$) ranging from 0.4 to 0.9 (e.g., Table 1, Case L). For values of $\phi < 0.4$, error rates declined with the lowest error rates observed when $\phi = 0$ (i.e., no autocorrelation in either time series). In the latter case, the average error rates across N for $\alpha = 0.02$, 0.05, and 0.10 were 0.015, 0.040, and 0.086, respectively. In other words, the Modified Chelton method was slightly conservative at all α values when little or no autocorrelation was present. We also simulated cases where X and Y had different parameters (i.e., $\phi_X \neq \phi_Y$) ranging from 0 to 0.9; Type I error rates turned out to be very similar to the case where $\phi_X = \phi_Y$ (e.g., Case L). Thus, if AR(1) models with positive coefficients ϕ are a reasonable description of the time series being correlated (as they frequently are in fisheries research), then it appears that the Modified Chelton method using $N/5$ lags j provides a robust procedure for testing correlations (Case L).

However, it is possible that this method may not perform as well for other time series models that have different autocorrelation functions than AR(1) models. We therefore simulated additional models that often depict time series encountered in practice (see Box and Jenkins (1976) for details of the various models described below). For example, we simulated both X and Y using AR(2) models (i.e., $X_t = \phi_1 X_{t-1} + \phi_2 X_{t-2} + \varepsilon_t$) with values of ϕ_1 ranging from 0.2 to 0.7 (in increments of 0.1) and ϕ_2 ranging from 0.2 to $(0.9 - \phi_1)$ (in increments of 0.1). Again, Type I error rates of the standard significance test were typically much larger than the specified α (Table 1, Case N), while error rates for the Modified Chelton method were reasonably accurate and precise (Case P). For $\alpha = 0.05$ and 0.10, error rates for the Modified Chelton method were at or below 0.055 and 0.12, respectively, except in extreme cases when $\phi_1 + \phi_2 = 0.9$. Results were similar for simulations where values of ϕ_1 and ϕ_2 differed between X and Y.

We examined an additional set of AR(2) models with ϕ_1 ranging from 0.8 to 1.8 (in increments of 0.2) and $\phi_2 = -\phi_1/2$. These models are of interest because they produce time series with apparent "cyclic" patterns (Box and Jenkins 1976, p. 62). A number of fish stocks in the Myers et al. (1995*b*) compendium of stock–recruitment data had autocorrelation functions of stock–recruitment residuals similar to the theoretical autocorrelation functions of these AR(2) models, namely, with large positive autocorrelations over the first several lags leading to negative autocorrelations at higher lags. For simulations with these models, the standard inference test once again gave large Type I error rates (Table 1, Case Q). In contrast, error rates for the Modified Chelton method were generally conservative (Case R). These conservative error rates were likely caused by the predominance of negative autocorrelations at higher lags. Because the autocorrelation estimator (eq. 7) is biased low, estimates of negative autocorrelations were slightly larger on average than their theoretical values. This resulted in lower values of N^*, and hence more conservative error rates, than were observed for the AR(1) and AR(2) models examined above.

We also examined a wide range of first- and second-order moving average models (MA(1) and MA(2) models) and mixed autoregressive moving average models (ARMA(1,1)) that produce time series with positive autocorrelations. However, in all cases, Type I error rates were roughly contained within the ranges observed for AR(1) models (i.e., Table 1, Case L). Furthermore, we simulated X and Y using various combinations of the AR(1) and AR(2) models described above, as well as models with moving average terms, and again found that averages and ranges of Type I error were consistent with those reported for Cases L, P, and R in Table 1.

Finally, we simulated a class of nonstationary time series to examine the performance of the Modified Chelton method under extreme conditions. As noted above, expressions for N^* are not valid for nonstationary data; however, for short time series, it may be difficult to distinguish between stationary and nonstationary processes. Specifically, we used ARIMA(1,1,0) models (i.e., $X_t = (1 + \phi)X_{t-1} - \phi X_{t-2} + \varepsilon_t$) with ϕ ranging from 0.0 (i.e., a random walk) to 0.9 (in increments of 0.3). The time series generated from such models will typically exhibit strong trends and high levels of autocorrelation. For values of ϕ ranging from 0.0 to 0.6, error rates of the standard inference test were extremely high (Table 1, Case S), yet the Modified Chelton method performed well, particularly at $\alpha = 0.02$ and 0.05 (Case T). However, when $\phi = 0.9$ (extreme trends, approaching an ARIMA(0,2,0) model), error rates for the standard test were greater than 0.7 for all combinations of N and α, while error rates of the Modified Chelton method were now well above the specified α levels (Case U).

In summary, results for the standard significance test of a sample correlation clearly illustrate that Type I error rates can be considerably greater than the specified α value when autocorrelation in the data is not taken into account. Unfortunately, there have been many published cases where correlation analyses were conducted but no adjustments were made for autocorrelation. Moreover, in cases where the testing procedure has been modified, the most commonly used method was that of Garrett and Petrie (1981), which we have shown to perform poorly at controlling Type I error rates. Nevertheless, our simulations showed that a modified version of the method of Chelton (1984) (or similarly, the method of Kope and Botsford (1990)) may often provide a useful procedure for testing sample correlations in the presence of positive autocorrelation. Specifically, we recommend that researchers use eq. 1 without the weighting function, with autocorrelations estimated over $N/5$ lags j using eq. 7, and with critical value (2) using $N^* - 2$ degrees of

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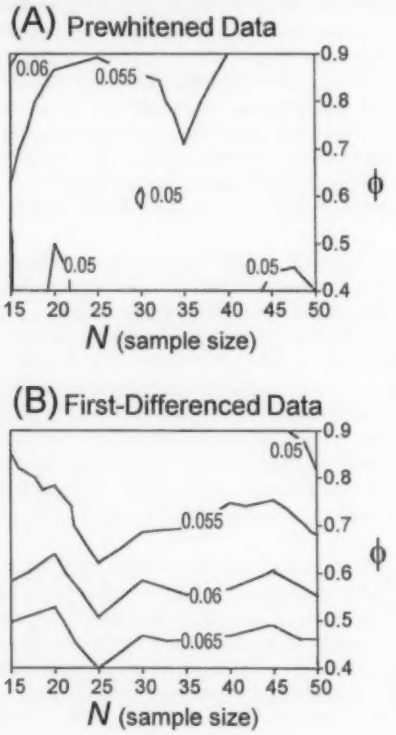
Fig. 3. Isopleths of simulated Type I error rates at $\alpha = 0.05$ for prewhitening and first-differencing. See text for details.



freedom. Although we used a limited set of stationary models in our simulations, they will likely represent many of the time series encountered in practice. Using more highly parameterized models could give different results, depending on how different their autocorrelation functions are in comparison with those examined here, but in most cases, such models will have limited biological relevance.

Prewhitening, first-differencing, and smoothing

The previous section compared methods of adjusting the test procedure for sample correlations, but as an alternative, many researchers remove autocorrelation from their data. One common approach is to prewhiten the time series (e.g., Milicich et al. 1992; Quinn and Niebauer 1995), i.e., fit an appropriate time series model to each data set and use the residuals for computing correlations. An example is to fit an AR(1) model to time series X to give the prewhitened series X_P:

$$(8) \qquad X_{P_t} = X_t - \hat{\phi}_X X_{t-1}.$$

Another very simple approach that is often effective at removing autocorrelation is to first-difference the time series (e.g., Thompson and Page 1989):

$$(9) \qquad \nabla X_t = X_t - X_{t-1}$$

where ∇X denotes the new, first-differenced time series. Note that first-differencing is equivalent to an extreme case of prewhitening where ϕ_X of the AR(1) model is equal to 1 (i.e., random walk), in which case, the process would be nonstationary (Box and Jenkins 1976, p. 56). Indeed, as discussed later, researchers have often first-differenced recruitment or environmental data prior to correlation analyses when these data exhibited strong time trends indicative of nonstationary time series.

To illustrate the potential effectiveness of prewhitening and first-differencing to control Type I error rates in tests of correlations, we used the baseline simulation procedure outlined above where AR(1) models were used to generate hypothetical data. Simulated time series were either prewhitened (by fitting AR(1) models to the data) or first-differenced, and then, correlations were computed between the new time series and tested using the standard significance test. Both procedures were very effective over the range of N and ϕ used in the simulations, as shown in Fig. 3 for $\alpha = 0.05$. Error rates for first-differencing did increase somewhat at low ϕ (Fig. 3B), where this procedure created moderate levels of negative lag-1 autocorrelation in the new time series, suggesting that first-differencing is most appropriate when high levels of autocorrelation are observed.

However, our concern in this section is not with Type I error rates but rather with how removing autocorrelation may affect Type II error rates in comparison with adjusting the test procedure. Specifically, removing autocorrelation (low-frequency variability) may limit a researcher's ability to detect the common effect of some slowly changing variable on fish population dynamics. This is quite important given the number of recent papers showing the presence of such slowly changing processes (e.g., Beamish 1995). Removing autocorrelation makes one or more implicit assumptions that low-frequency variability in time series (*i*) is unimportant, (*ii*) is not common between the two data series, (*iii*) potentially obscures the detection of common and more important high-frequency variability, or (*iv*) is caused by the same factors that cause high-frequency variability. However researchers rarely state such assumptions when removing autocorrelation and often do not seem to recognize the procedure's possible implications for their analyses. For example, if low-frequency variability is the dominant source of covariation between two time series, one would expect that removing autocorrelation from them, rather than adjusting the hypothesis testing procedure, may increase the Type II error rate (or equivalently, reduce statistical power (= 1 – Type II error rate)).

On the other hand, there may be cases where removing autocorrelation increases statistical power. This would be expected, for example, when high-frequency variability is the dominant source of covariation. Moreover, because autocorrelation can seriously reduce the degrees of freedom of a sample correlation, and hence limit statistical power, removing autocorrelation may increase power even when low-frequency variability is an important source of covariation by "restoring" degrees of freedom. In addition, it may be necessary to remove autocorrelation when there is a priori reason to believe that low-frequency patterns of variability may be spurious (e.g., Thompson and Page 1989).

In some cases, researchers who are particularly interested in patterns of low-frequency variability in fish data will

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smooth time series (e.g., Drinkwater and Myers 1987; Hollowed et al. 1987). Smoothing is an opposite approach to prewhitening or first-differencing because it removes high-frequency rather than low-frequency variation. In contrast with the vague assumptions often associated with prewhitening or first-differencing, smoothing is usually applied in correlation analyses with the clear assumption that high-frequency "noise" such as measurement error may obscure detection of common, important low-frequency variability. As an example of this approach, consider a simple two-point running mean:

$$(10) \quad X_{S_t} = (X_t + X_{t-1})/2$$

where X_S denotes the new, smoothed time series. Note that while smoothing can improve the detection of synchronous low-frequency variability (Davis 1977), it will increase autocorrelation in the time series and thus increase the probability of Type I error if the test procedure is not adjusted to account for the autocorrelation.

In the following section, we use a theoretical example to examine some of the possible implications of prewhitening, first-differencing, and, for comparison, smoothing time series in correlation analyses. In particular, we examine the extent to which these transformations can alter real correlations and affect the statistical power to detect them in comparison with using the original time series and the Modified Chelton test procedure.

Methods—prewhitening, first-differencing, and smoothing

Several researchers have attempted to generate hypotheses about sources of environmental variation on recruitment of fish stocks by testing whether separate stocks show covariation among themselves or with environmental variables (e.g., Koslow et al. 1987; Thompson and Page 1989). Consider the following simple model for how a correlation might arise between a pair of time series X and Y, where each is a linear combination of an "independent" source of variability, I_X and I_Y respectively, and a "common" source of variability, C:

$$(11) \quad \begin{aligned} X_t &= I_X + aC_t \\ Y_t &= I_Y + bC_t \end{aligned}$$

where a and b are constants and I_X, I_Y, and C are independent of one another (i.e., uncorrelated). One could think of X and Y as time series of recruitment or environmental data that are both influenced by some unobservable, underlying environmental process C. We are interested in the case where each of the independent and common sources of variability is autocorrelated to some extent. Thus, we consider the simple case where I_X, I_Y, and C are each defined as an AR(1) process (eq. 4) with autoregressive parameters ϕ_{I_X}, ϕ_{I_Y}, and ϕ_C, respectively.

Intuitively, the greater the constants a and b (or variance of C), the greater the correlation will be between X and Y. Furthermore, larger values of ϕ_{I_X}, ϕ_{I_Y}, and ϕ_C will obviously result in greater autocorrelation in X and Y. However, when ϕ_C is large and ϕ_{I_X} and ϕ_{I_Y} are small, removing autocorrelation should also remove much of the common signal associated with C, thereby reducing the correlation between X and Y. The opposite should be true when ϕ_C is small and ϕ_{I_X} and ϕ_{I_Y} are large. We investigated the potential magnitude of these effects in the following ways.

Given eq. 11, we derived formulas for the theoretical asymptotic (large sample size) correlations between X and Y for the original time series and for series that were prewhitened via eq. 8, first-differenced (eq. 9), and smoothed (eq. 10) (see Appendix).

To examine the effects of prewhitening, first-differencing, and smoothing on the statistical power to detect real correlations, we used Monte Carlo simulations. Specifically, we simulated eq. 11 with the variances of I_X, I_Y, and C all held constant at 1. Simulations were done for $N = 20$ and $N = 50$. In each case, we generated time series of X and Y, with positive correlations between them, using values of a and b (i.e., $a = b$) from 0.4 to 2 (in increments of 0.2), values of ϕ_I (i.e., $\phi_{I_X} = \phi_{I_Y}$) from 0 to 0.9 (in increments of 0.1), and values of ϕ_C from 0 to 0.9 (in increments of 0.1). For each combination of (a,b), ϕ_I, and ϕ_C, 2000 Monte Carlo trials were done in which we computed sample correlations between X and Y at lag zero and tabulated statistical power (i.e., the proportion of cases where the null hypothesis of no correlation was rejected for a two-tailed test with $\alpha = 0.05$) for the following methods: (A) using the original (autocorrelated) time series and the Modified Chelton test procedure outlined above, (B) prewhitening each time series using AR(1) models and then using the standard inference test, (C) first-differencing each time series and the standard inference test, and (D) smoothing each time series using a two-point running mean and the Modified Chelton method.

Results and discussion—prewhitening, first-differencing, and smoothing

As demonstrated in formulas in the Appendix, prewhitening and, in particular, first-differencing can substantially reduce the asymptotic correlation (ρ_{XY}) between X and Y when the primary source of autocorrelation is also the source of covariation (i.e., $\phi_C > \phi_I$). For example, Table 2 presents asymptotic correlations for four combinations of ϕ_I and ϕ_C when $(a, b, \sigma_{I_X}, \sigma_{I_Y}, \sigma_C) = 1$. For the extreme case where $\phi_I = 0$ and $\phi_C = 0.9$, prewhitening and first-differencing will reduce ρ_{XY} from 0.50 to only 0.25 and 0.09, respectively. In contrast, smoothing will increase ρ_{XY} from 0.50 to 0.66. However, when independent sources of variability are the primary sources of autocorrelation ($\phi_I > \phi_C$), prewhitening and first-differencing will increase ρ_{XY}, while smoothing reduces it (right side of Table 2).

In the case where $\phi_C > \phi_I$, it is conceivable that reductions in correlations caused by prewhitening or first-differencing may be offset by the increase in degrees of freedom obtained by removing autocorrelation, which could increase or at least maintain statistical power. However, our simulations showed that in many cases, statistical power was reduced substantially. Figure 4 presents isopleths of changes in statistical power for three combinations of N and (a,b) when X and Y were either prewhitened, first-differenced, or smoothed. Positive values indicate increases in statistical power, while negative values (shaded areas in Fig. 4) indicate decreases. For example, a value of –0.2 means that out of 100 tests, there would be 20 *fewer* cases in which the null hypothesis was correctly rejected compared with using the

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Table 2. Theoretical asymptotic correlations, computed from equations in Table A (see Appendix), between time series X and Y for the original time series, prewhitened series, first-differenced series, and smoothed series.

Transformation of data	Asymptotic correlation between X and Y (ρ_{XY})			
	$\phi_I = 0.0$		$\phi_C = 0.0$	
	$\phi_C = 0.6$	$\phi_C = 0.9$	$\phi_I = 0.6$	$\phi_I = 0.9$
(A) Original	0.50	0.50	0.50	0.50
(B) Prewhitened	0.40	0.25	0.60	0.75
(C) First-differenced	0.29	0.09	0.71	0.91
(D) Smoothed	0.62	0.66	0.38	0.34

Note: $(a,b,\sigma_{I_x},\sigma_{I_y},\sigma_C) = 1$ and $\phi_{I_x} = \phi_{I_y} = \phi_I$.

original time series and the Modified Chelton test procedure. (Note that eqs. A1 and A3 in the Appendix provide a rough indication of the levels of covariation and autocorrelation in the original time series X and Y for given values of (a,b), ϕ_I, and ϕ_C.)

For small values of (a,b) (i.e., small effect of the common factor C), prewhitening and first-differencing increased statistical power in most cases, especially for $\phi_I >> \phi_C$ (e.g., $N = 20$ and $(a,b) = 0.6$; top panels in Fig. 4). However, the situation changed appreciably when the common factor was a more important source of variability in X and Y (e.g., $N = 20$ and $(a,b) = 1.4$; middle panels in Fig. 4). Here, prewhitening and first-differencing resulted in reductions in power by as much as 0.2 or 0.3 for combinations where $\phi_C > \phi_I$ and resulted in increases in power that were greatest when both ϕ_I and ϕ_C were large. Larger changes in power were observed for given values of (a,b) when $N = 50$ (e.g., $N = 50$ and $(a,b) = 1.0$; bottom panels in Fig. 4).

The patterns shown in Fig. 4 for prewhitening and first-differencing were typical of the range of combinations of N and (a,b) that we explored. In general, these transformations increased power when $\phi_I > \phi_C$ by increasing degrees of freedom and levels of covariation. However, when $\phi_C > \phi_I$, removing autocorrelation typically reduced power despite increasing degrees of freedom because these procedures resulted in often large reductions in covariation.

Interestingly, smoothing using a two-point running mean increased power only slightly when $\phi_C >> \phi_I$ (Fig. 4). For most combinations where $\phi_C > \phi_I$, increases in covariation due to smoothing were offset by decreased degrees of freedom (through increased autocorrelation), resulting in reduced power. Using three-, four-, or five-point running means also resulted in only slight increases in power when $\phi_C >> \phi_I$.

Thus, as illustrated by this theoretical example, prewhitening and, in particular, first-differencing can remove much of the covariation between autocorrelated time series and consequently reduce the power to detect it *when* the source of that covariation is also the main source of the autocorrelation. Of course, the opposite is also true. That is, if autocorrelation is due in large part to variability that is unique to a given time series, then prewhitening or first-differencing can substantially increase power. It is not our purpose to present an exhaustive examination of the advantages or disadvantages of such methods as prewhitening, first-differencing, or smoothing, but rather to point out that such transformations may have unintended effects that alter the interpretation of analyses. By using prewhitening or first-differencing, past researchers may have missed detecting some environmental effect or some correlation among stocks that existed due to the common effect of some important but slowly changing variable.

A final frequently used method of treating time series in fisheries is to remove time trends. Recruitment and environmental time series often have distinct increasing or decreasing trends over time (e.g., Cohen et al. 1991) that could easily produce misleading correlations between the series when these trends are independent (Plosser and Schwert 1978) or could mask covariation at higher frequencies (Kope and Botsford 1990). Furthermore, strong trends indicate nonstationary time series, in which case, methods for adjusting the test procedure may not adequately control type I error rates. Thus, some researchers recommend removing time trends before computing correlations by either first-differencing (e.g., Cohen et al. 1991) or "detrending" the data (typically using residuals from a linear regression versus time) (e.g., Botsford and Brittnacher 1992). Once again, however, such practices may remove important information about a possible *common* mechanism influencing different data series. By removing time trends, we are assuming that they are unrelated, yet there are obvious mechanisms that could produce common time trends among recruitment data such as trends in environmental variables, habitat degradation, or trends in the abundances of competitor, prey, or predator species (e.g., Butler 1991). In addition, short time series can often appear to have deterministic or nonstationary trends, even though the series are stationary and only moderately autocorrelated. Thus, even though we may not be able to distinguish between time trends that are caused by a common mechanism and those that are not, researchers should consider both possibilities when choosing methods of analysis and they should interpret their results cautiously.

Empirical example: sockeye salmon of Bristol Bay, Alaska

The previous section quantified potential changes in correlations and statistical power that could be caused by prewhitening, first-differencing, and smoothing. Here, we briefly illustrate the possible effects of these transformations using recruitment data for four stocks of sockeye salmon from Bristol Bay, Alaska. There has been growing interest in the low-frequency variability, often referred to as interdecadal variability, of physical and biological processes in the North Pacific and their effects on the productivity of fish stocks such as Bristol Bay sockeye (e.g., Hare and Francis 1995; Adkison et al. 1996).

Methods—empirical example

We selected four stocks of Bristol Bay sockeye that had autocorrelated indices of survival rate: the Igushik, Kvichak, Ugashik, and Wood River stocks. For each stock, we used abundances of spawners and total recruits for 34 brood years from 1956 to 1989 (B. Cross, Alaska Department of Fish and Game, Anchorage, Alaska, personal communication). Using these data, we computed an index of survival rate (SR

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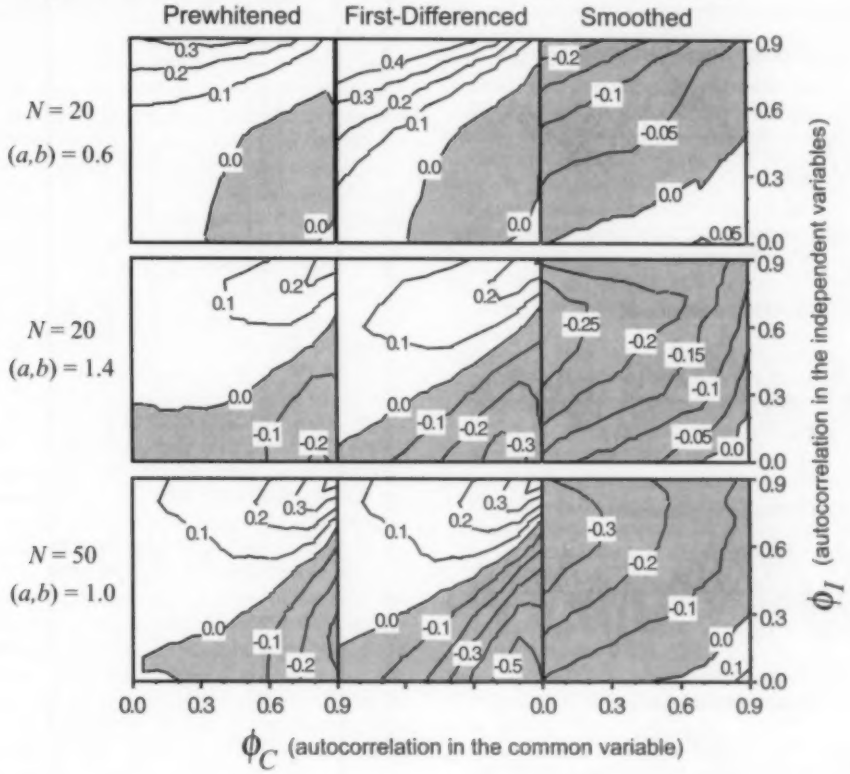
Fig. 4. Isopleths of changes in statistical power for prewhitening, first-differencing, and smoothing compared with using the original time series and the Modified Chelton method for three sets of conditions of N and (a,b). Positive values indicate increases in statistical power over using the original time series, while negative values indicate decreases. See text for details.



index) for each stock to account for changes in spawner abundance, within-stock density dependence, and lognormal error structure. This index was the time series of residuals from the fit of the Ricker stock–recruitment relationship, i.e., linear regression of $\log_e$(recruits per spawner) versus spawners. The autocorrelation and partial autocorrelation functions of the four SR indices were consistent with AR(1) processes, with estimates of lag-1 autocorrelation ranging from 0.44 to 0.70. We therefore fit AR(1) models to these SR indices as an example of prewhitening.

We computed pairwise correlations among the four SR data series using the original, prewhitened, first-differenced, and smoothed data (two-point running mean). Standard hypothesis tests (two-tailed, $\alpha = 0.05$) were used for correlations among prewhitened or first-differenced data, while the Modified Chelton method was used for correlations among the original or smoothed data. Statistical power was estimated for each pairwise comparison using the sample correlation and the equations given in Zar (1984, p. 312). Because these "analytical" equations are for serially independent data, they provide only a rough approximation of power for comparisons among the original and smoothed (i.e., autocorrelated) data. However, in our simulation analyses described above, we found that the analytical estimates of power consistently underestimated the actual, simulated power by roughly 0.05 on average for cases where levels of covariation and autocorrelation were similar to those observed here for the four Bristol Bay sockeye stocks. Thus, the estimates of power provided below for the original and smoothed data are likely slight underestimates of the actual statistical power.

Results and discussion—empirical example

The time series of SR indices and their transformations are shown in Fig. 5. Extended periods of negative residuals (1960s) and positive residuals (1970s) in each of the original SR indices provide evidence that *synchronous* low-frequency variability is an important feature of these data (Fig. 5A), suggesting a shared influence of some autocorrelated environmental factor. Prewhitening and, in particular, first-differencing appeared to remove much of the coherence between the SR indices (Figs. 5B and 5C). In contrast, using a two-point running mean to smooth the SR indices accentuated the low-frequency trends and the apparent coherence of the time series (Fig. 5D).

Correlations among the original and transformed SR indices (Table 3) reflect the patterns observed in Fig. 5. For example, prewhitening and first-differencing reduced the *average* correlation among the SR series from 0.50 to only 0.24 and 0.11, respectively (last row of Table 3), and reduced the number of significant correlations from 4 to 2 and 1, respectively. Moreover, prewhitening and, to a greater extent, first-differencing reduced the average statistical power from 0.5 to 0.34 and 0.18, respectively, despite having twice

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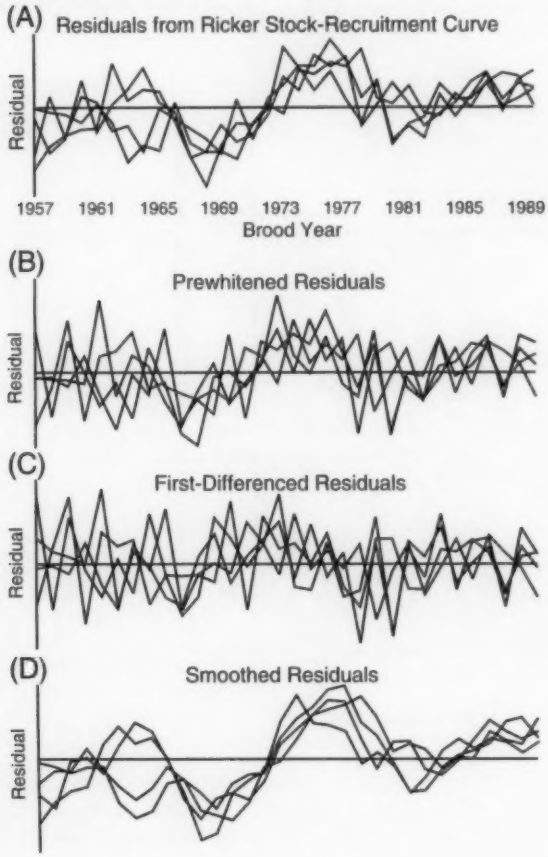

Fig. 5. Time series for four Bristol Bay sockeye stocks of (A) residuals from the Ricker stock–recruitment curve (i.e., survival rate (SR) indices), (B) prewhitened SR indices, (C) first-differenced SR indices, and (D) smoothed SR indices.

the number of degrees of freedom as the average comparison among the original, autocorrelated SR indices (i.e., 31 versus an average of 14 degrees of freedom) (right half of Table 3). In contrast, smoothing increased the average correlation to 0.62, although statistical power changed little because of the corresponding decreases in degrees of freedom (Table 3).

Thus, this example represents a case where the researcher could easily miss detecting the shared influence of a slowly changing process on fish productivity by removing autocorrelation rather than adjusting the test procedure to account for autocorrelation. The tendency for correlations to be lower among prewhitened or first-differenced data and higher among smoothed data suggests that low-frequency components of variability were the dominant source of covariation in the original SR indices.

Conclusions

A growing body of literature documents the importance of slow changes, or low-frequency variability, in recruitment of fish species and in the physical and biological processes that

Table 3. Sample correlations and corresponding statistical power for comparisons among the survival rate (SR) indices of four Bristol Bay sockeye stocks: Igushik (IGU), Kvichak (KVI), Ugashik (UGA), and Wood (WOO).

	Effective degrees of freedom (N^* – 2)		Sample correlation coefficient				Statistical power			
Comparison	Original	Smoothed	Original	Prewhitened	First-differenced	Smoothed	Original	Prewhitened	First-differenced	Smoothed
(1) IGU vs. KVI	11	7	0.62*	0.43*	0.32	0.73*	0.65	0.70	0.44	0.64
(2) IGU vs. UGA	13	9	0.41	0.11	–0.02	0.52	0.31	0.08	0.02	0.35
(3) IGU vs. WOO	13	7	0.55*	0.28	0.14	0.68*	0.58	0.36	0.12	0.53
(4) KVI vs. UGA	21	13	0.33	0.05	–0.10	0.43	0.32	0.04	0.01	0.38
(5) KVI vs. WOO	16	8	0.51*	0.44*	0.35*	0.59	0.60	0.73	0.51	0.43
(6) UGA vs. WOO	12	7	0.56*	0.16	–0.06	0.76*	0.56	0.14	0.01	0.72
Average	**14**	**9**	**0.50**	**0.24**	**0.11**	**0.62**	**0.50**	**0.34**	**0.18**	**0.51**

Note: *Statistically significant ($P < 0.05$).

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may affect recruitment (e.g., Hollowed and Wooster 1992). However, in an increasing number of applications in fisheries and ecological literature, autocorrelation (low-frequency variability) is removed from time series before correlation analyses are conducted in an effort to control Type I error rates (e.g., Milicich et al. 1992; Meekan et al. 1993; Robertson et al. 1993). We have shown that a specific method for adjusting the test procedure of a sample correlation may often provide a useful alternative to removing autocorrelation. Our theoretical and empirical examples showed that statistical power can be significantly greater for this adjustment method in comparison with removing autocorrelation when low-frequency variability is the major source of covariation, as many researchers are finding (e.g., Beamish 1995).

It is therefore important that fisheries researchers, when dealing with autocorrelated data, generate hypotheses about what time scales of variability in environmental processes are important. Given such hypotheses, researchers can then select appropriate tools for investigating patterns of covariation at these different time scales (e.g., Hollowed et al. 1987). For example, if high-frequency (i.e., rapid time scale) components of variability are the dominant source of covariation, then prewhitening (or first-differencing) may be useful because it should increase covariation and the statistical power to detect it by removing independent, low-frequency variability that obscures the detection of that covariation. Conversely, smoothing of some form may be useful for detecting low-frequency (slow time scale) sources of covariation, although correlations must be tested using the adjustment method outlined above to account for autocorrelation that will be present in the smoothed data. In any of these cases, researchers should be aware and cautious of the distorting effects that these various transformations can have on data.

While the concerns we have raised in this paper relate to simple correlation analysis, autocorrelation has similar implications for many other statistical methods such as regression (e.g., Bence 1995).

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Appendix

Based on eq. 11, we derived theoretical expressions for the asymptotic correlation between the original time series of X and Y and between prewhitened, first-differenced, and smoothed versions of X and Y by evaluating the variances and covariances of these series (e.g., Kalbfleisch 1985, pp. 176–179). For example, it can be shown that the asymptotic Pearson product-moment correlation between the *original* time series of X and Y as defined by eq. 11 is

$$\text{(A1)} \quad \rho_{XY} = \frac{\text{cov}(X,Y)}{\sigma_X \sigma_Y} = \frac{ab\sigma_C^2}{\sigma_X \sigma_Y}$$

where

$$\text{(A2)} \quad \sigma_X^2 = \sigma_{I_X}^2 + a^2\sigma_C^2$$

and similarly for σ_Y^2.

This asymptotic correlation is not affected by the presence of autocorrelation in either I_X, I_Y, or C. However, when I_X, I_Y, and C are defined as AR(1) processes as in eq. 4 (with first-order autoregressive parameters σ_{I_X}, σ_{I_Y}, and ϕ_C, respectively), it can be shown that X will have an asymptotic autocorrelation function

$$\text{(A3)} \quad \rho_{XX}(j) = \frac{\sigma_{I_X}^2 \phi_{I_X}^{|j|} + a^2\sigma_C^2 \phi_C^{|j|}}{\sigma_X^2}$$

and similarly for Y. Note that the shape of the autocorrelation function defined by eq. A3 is similar but not identical to that of an AR(1) process (eq. 5) except when $\phi_{I_X} = \phi_C$.

To examine how prewhitening, first-differencing, and smoothing could affect the asymptotic correlation between X and Y, we used eq. 8 (prewhitening using an AR(1) model), eq. 9 (first-differencing), and eq. 10 (smoothing using a two-point running mean) to transform the series. When fitting AR(1) processes to X and Y, the first-order auroregressive parameters would, asymptotically, be equal to $\rho_{XX}(1)$ and $\rho_{YY}(1)$ as defined by eq. A3. For simplicity, we denote these parameters as ϕ_X and ϕ_Y.

The asymptotic correlations between the prewhitened, first-differenced, and smoothed times series of X and Y are shown in Table A. First, note that these correlations equal the original correlation (eq. A1) when there is no autocorrelation present (i.e., ϕ_{I_X}, ϕ_{I_Y}, and ϕ_C equal zero, and hence, ϕ_X and ϕ_Y also equal zero). However, if the common variable C is autocorrelated (i.e., $\phi_C > 0$), then the correlations between X and Y are *less* than the original correlation for both prewhitening and first-differencing and *greater* for smoothing. Larger values of ϕ_C result in larger differences in the correlations. The opposite is true if I_X and I_Y are autocorrelated but C is not. That is, prewhitening and first-differencing increase the correlation between X and Y, while smoothing reduces it.

Table A. Asymptotic correlations between prewhitened, first-differenced, and smoothed time series of X and Y (eq. 11) when I_X, I_Y, and C are defined as AR(1) processes.

Method of transformation	Asymptotic correlation between X and Y	Variances of X and Y
Prewhitening (eq. 8)	$\rho_{X_p Y_p} = \dfrac{ab\sigma_C^2(1 - \phi_C\phi_X - \phi_C\phi_Y + \phi_X\phi_Y)}{\sigma_{X_p}\sigma_{Y_p}}$	$\sigma_{X_p}^2 = \sigma_{I_X}^2(1 - 2\phi_{I_X}\phi_X + \phi_X^2) + a^2\sigma_C^2(1 - 2\phi_C\phi_X + \phi_X^2)$ and similarly for $\sigma_{Y_p}^2$
First-differencing (eq. 9)	$\rho_{\nabla X \nabla Y} = \dfrac{2ab\sigma_C^2(1 - \phi_C)}{\sigma_{\nabla X}\sigma_{\nabla Y}}$	$\sigma_{\nabla X}^2 = 2\sigma_{I_X}^2(1 - \phi_{I_X}) + 2a^2\sigma_C^2(1 - \phi_C)$ and similarly for $\sigma_{\nabla Y}^2$
Smoothing (eq. 10)	$\rho_{X_s Y_s} = \dfrac{ab\sigma_C^2(1 + \phi_C)}{2\sigma_{X_s}\sigma_{Y_s}}$	$\sigma_{X_s}^2 = [\sigma_{I_X}^2(1 + \phi_{I_X}) + a^2\sigma_C^2(1 + \phi_C)]/2$ and similarly for $\sigma_{Y_s}^2$

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Effects of habitat isolation on the recovery of fish assemblages in experimentally defaunated stream pools in Arkansas

David George Lonzarich, Melvin L. Warren, Jr., and Mary Ruth Elger Lonzarich

Abstract: We removed fish from pools in two Arkansas streams to determine recolonization rates and the effects of isolation (i.e., riffle length, riffle depth, distance to large source pools, and location), pool area, and assemblage size on recovery. To determine pool-specific recovery rates, we repeatedly snorkeled 12 pools over a 40-day recovery period. Results indicated the effects of isolation on percent numerical recovery, but no effects of pool area or assemblage size. Numerical recovery of assemblages in pools separated from neighboring pools by short riffles occurred by day 30 whereas more isolated pools had not reached 70% numerical recovery by day 40. Recovery also was more rapid in downstream pools and in pools that were closer to large source pools. Finally, recovery patterns differed among species and size-classes, with large fish (<100 mm total length) recolonizing pools more rapidly than small fish. This is the first study to quantify species- and assemblage-level recolonization rates at the scale of individual pools and the effects of isolation on recovery. The findings of this study have potentially important implications for research aimed at understanding the ecology of stream fishes and predicting the consequences of land-use activities.

Résumé : Nous avons recueilli des poissons dans certaines des fosses de deux cours d'eau de l'Arkansas pour évaluer la vitesse de repeuplement et les effets de l'isolement (c.-à-d. longueur et profondeur des rapides, distance jusqu'aux grandes fosses d'origine et emplacement), de la superficie de la fosse et de la taille des assemblages sur le repeuplement. Pour déterminer la vitesse de repeuplement en fonction de la fosse, nous avons inspecté en plongée libre 12 fosses à plusieurs reprises durant une période de 40 jours. D'après les résultats obtenus, nous avons conclu que l'isolement influe sur le repeuplement numérique, tandis que la superficie de la fosse et la taille des assemblages n'ont pas d'effet. La reconstitution numérique des assemblages était complète au 30^e jour dans les fosses séparées des autres fosses par de courts rapides; dans les fosses plus isolées, elle n'avait pas atteint 70 % au 40^e jour. Nous avons aussi constaté que le repeuplement était plus rapide dans les fosses d'aval, ainsi que dans celles qui se trouvaient plus près des grandes fosses d'origine. Par ailleurs, le mode de repeuplement diffère selon l'espèce et la classe de taille, les poissons de grande taille (longeur totale <100 mm) repeuplant les fosses plus rapidement que les poissons de petite taille. C'est la première fois qu'on quantifie le repeuplement en fonction de l'espèce et de la taille des assemblages dans des fosses individuelles et qu'on évalue l'effet de l'isolement. Les résultats de nos travaux pourraient être d'une grande utilité pour l'étude de l'écologie des poissons des milieux lotiques et pour la prévision des conséquences des différentes formes d'utilisation des sols.

[Traduit par la Rédaction]

Introduction

Episodic disturbances, such as floods and droughts, contribute significantly to the organization and stability of stream communities by affecting the abundance and distribution of fishes (Meffe 1984; Erman et al. 1988; Pearsons et al. 1992; Strange et al. 1992). The rate of community recovery from these disturbances will depend strongly on how rapidly different fish species recolonize disturbed stream segments. A large body of evidence indicates that recolonization of disturbed stream segments by fishes can be fairly rapid (<1 year, e.g., Niemi et al. 1990). Recovery of fish assemblages in short reaches and habitat units can occur on a scale of days and weeks (Peterson and Bayley 1993; Sheldon and Meffe 1995). Nevertheless, research thus far has revealed little about physical factors that contribute to recovery, especially at small spatial scales (Detenbeck et al. 1992).

One significant shortcoming of research on fish recolonization is the typical use of invasive postdisturbance sampling methods that permanently remove fish from surveyed habitats and thereby alter natural recovery patterns. This approach precludes use of repeated sampling of defaunated habitats that is necessary to accurately determine rates of recovery associated with different habitat conditions (e.g., habitat size, habitat complexity). Sheldon and Meffe (1995)

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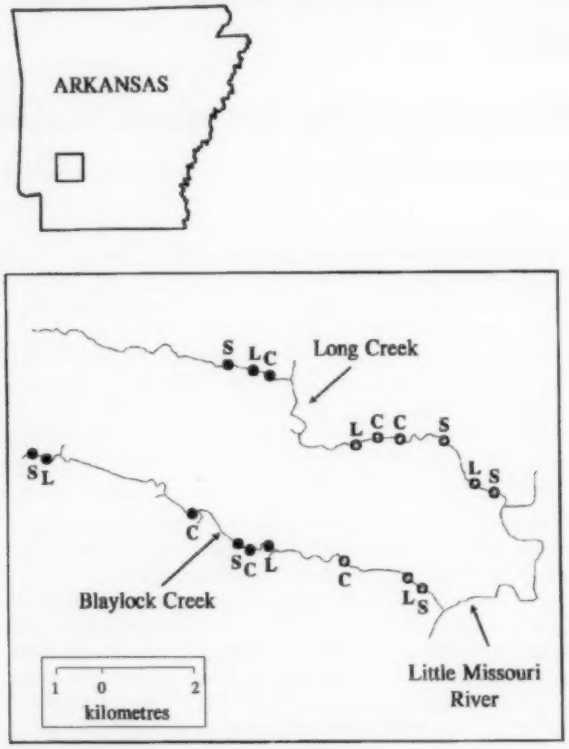
Fig. 1. Location of 12 experimental and 6 control pools in Long and Blaylock creeks. Upstream pools are represented by solid circles and downstream pools by open circles.



speculated that differences in fish recolonization rates in upstream and downstream pools may have been due to distance from source populations or differences in habitat characteristics. Their study provided insights into the mechanisms that shape patterns of recolonization, but many questions about the ecology of fish recolonization and resilience of fish communities remain unanswered. For example, will fish assemblages in small habitat units recover more rapidly than assemblages in large habitats by virtue of differences in the size of assemblages? Is colonization influenced by the degree to which stream habitats are isolated from one another, as would be predicted by island biogeography theory (MacArthur and Wilson 1967)?

Identifying the effects of habitat disturbance on biotic recovery would at the very least require comparisons of recovery rates across habitats that differ in quality. However, we suspect that it also important to examine the influence on recovery of the spatial distribution of habitats in specific stream segments. The size and distribution of habitats (e.g., pools, riffles) in a stream segment may influence faunal recovery in disturbed areas by influencing rates of movement between equivalent habitat types (e.g., pools) and the composition of potential colonists. Many studies have examined the consequences of land-use activities on habitat quality and the ecology of stream fishes (e.g., Shirvell 1990; Hicks et al. 1991; Lonzarich and Quinn 1995). By contrast, little attention has been given to the ecological effects of habitat isolation (but see Gerking 1953) even though the distribution of habitats can be altered by land-use activities (Karr and Schlosser 1978). One potentially important consequence of changes in the spatial distribution of habitats may lie in the ability of fish to track resources and move among suitable habitat types (e.g., pools). In gaining access to suitable habitats, pool-dwelling fishes may need to cross habitats, such as shallow-water riffles, that can either have high energy costs or pose high predation risks (Harvey and Stewart 1991; Lonzarich and Quinn 1995). Because the ability of fish to track changes in resources will be influenced by the costs associated with movement, the distance between habitats or the depth of corridors may contribute significantly to patterns of assemblage recovery.

In this study, we examine patterns of recolonization of fishes in experimentally defaunated stream pools standardized to evaluate the effects of isolation on faunal recovery. To develop estimates of recovery rates for individual pools and habitat conditions, we conducted repeated snorkeling surveys of experimental pools over a 40-day period. Our experimental design allowed us to evaluate effects on recovery of several factors contributing to isolation of a pool habitat: the distance between pool habitats, the depth of corridors between pool habitats (i.e., riffles), the distance to large "source" pools, and location in the drainage. Our primary objectives were to (*i*) estimate pool-scale recovery rates of fishes and (*ii*) evaluate the influences on recovery rates of pool isolation and two other potentially important factors: pool area and predisturbance assemblage size.

Materials and methods

Site selection

We carried out experiments between July and August 1995 in two tributary streams of the Little Missouri River in the Ouachita National Forest, Arkansas (34°22′30″ lat., 93°52′30″ long., Fig. 1). Long and Blaylock creeks are relatively short (<20 km), low-gradient systems that flow through forested and mountainous terrain. General characteristics of the streams included bedrock, cobble, and gravel substrates, dense riparian vegetation, and well-developed pool and riffle habitats. Previous research has shown that the proportion and average size of pool and riffle habitats in the two streams were very similar (Clingenpeel 1994). Likewise, fish assemblages in the two creeks were nearly identical when measured in terms of species composition, rank dominance, and densities (D.G. Lonzarich, unpublished data, Table 1). Based primarily on differences in the length of adjacent riffles, we selected 12 experimental pools in the two streams (six in each stream) and assigned them to one of two treatment categories: (*i*) short-riffle pools and (*ii*) long-riffle pools. Short-riffle pools were separated from the adjacent upstream and downstream pools by riffles <10 m (mean = 9.1 m) (Table 2). Long-riffle pools were separated from adjacent pools by riffles that were at least 20 m long, although this distance typically exceeded 35 m (mean = 46 m). Along with riffle length, we examined the effect of pool position on recovery by assigning the 12 pools into upstream and downstream categories (Fig. 1).

In addition to the 12 experimental pools, we randomly selected six pools as unmanipulated controls (Table 2). Although we did not remove fish from these pools, we monitored their assemblages in

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Table 1. Comparisons of fish densities (individuals/m^2) for target species in the two study streams.

Species	Blaylock Creek	Long Creek
Central stoneroller (*Campostoma anomalum*)	0.80	0.82
Striped shiner (*Luxilus chrysocephalus*)	0.47	0.56
Creek chub (*Semotilus atromaculatus*)	0.38	0.17
Orangebelly darter (*Etheostoma radiosum*)	0.16	0.16
Yellow bullhead (*Ameiurus natalis*)	0.06	0.04
Longear sunfish (*Lepomis megalotis*)	0.05	0.11
Northern studfish (*Fundulus catenatus*)	0.05	0.06
Bigeye shiner (*Notropis boops*)	0.03	0.03
Greenside darter (*Etheostoma blennioides*)	0.02	0.02
Smallmouth bass (*Micropterus dolomieu*)	0.02	0.03
Northern hog sucker (*Hypentelium nigricans*)	0.01	0.02
Redfin shiner (*Lythrurus umbratilis*)	0.01	0.01
Bluntnose minnow (*Pimephales notatus*)	*	
Green sunfish (*Lepomis cyanellus*)	*	
Blackspotted topminnow (*Fundulus olivaceus*)		*
Freckled madtom (*Noturus nocturnus*)		*
Logperch (*Percina caprodes*)		*
Rock bass (*Ambloplites rupestris*)		*
Slender madtom (*Noturus exilis*)		*
Total density	2.06	2.03

Note: Estimates are based on electrofishing surveys of 24 pools in each of the two streams conducted in summer 1994 (D.G. Lonzarich, unpublished data). An asterisk indicates that fewer than five individuals were collected at all sites combined.

the same way as for the experimental pools. We added controls to describe the natural dynamics of pool assemblages and to identify the presence of any temporal trends that could have influenced interpretations of biotic recovery in the experimental pools.

For each pool, we measured total length, width, area, maximum depth, substrate composition (boulder, cobble, gravel), distance to the stream mouth, and distance to the nearest large pool (>250 m^2). Biological characteristics measured included species richness, total assemblage, and individual species densities. With the exception of riffle length, the average physical dimensions, species richness, and fish densities of the riffle treatments were not different (Table 2, $P > 0.05$, two-sample t-test), although the statistical power of tests on physical habitat variables was generally low ($\beta > 0.90$). Typically, downstream pools were larger and bordered by deeper riffles than those upstream (Table 2, $P < 0.05$, two-sample t-test). On average, control pools were smaller and had higher fish densities than experimental pools.

Fish surveys

In this study, we focused on a subset of the fish species in the two streams. Because of concerns with sampling efficiency, we did not include small juvenile fishes (<25 mm) or bottom-dwelling species that often hide within the substrate and become difficult to locate using visual census techniques. Bottom-dwelling species not surveyed included northern hog sucker (*Hypentelium nigricans*), orangebelly darter (*Etheostoma radiosum*), greenside darter (*Etheostoma blennioides*), and yellow bullhead (*Ameiurus natalis*). Numerically, these species and small juveniles were a minor component of the pool assemblages in the study streams, generally accounting for <10% of all fish collected by electrofishing. Species collected in this study included central stoneroller (*Campostoma anomalum*), striped shiner (*Luxilus chrysocephalus*), redfin shiner (*Lythrurus umbratilis*), bigeye shiner (*Notropis boops*), northern studfish (*Fundulus catenatus*), creek chub (*Semotilus atromaculatus*), longear sunfish (*Lepomis megalotis*), and smallmouth bass (*Micropterus dolomieu*). Because the last three species ranged considerably in size, we distinguished two size categories: small (25–100 mm total length) and large fish (>100 mm total length).

We removed fish from experimental pools by isolating each pool with 6-mm-mesh block seines and sampling with a Smith–Root battery-powered, backpack electrofisher. Pools were then sampled between four and seven times until no fish were collected on two consecutive passes. When removed from experimental pools, individuals were either sacrificed or relocated up to 500 m downstream into pools below barriers that prevented upstream movement. We evaluated the effectiveness of the electrofishing technique in removing target species by conducting snorkeling surveys in the isolated pools immediately upon the completion of sampling. In snorkeling counts of six of the 12 experimental pools, we observed only three fish that belonged to the target group of species.

Snorkeling census

We censused fish in experimental pools by snorkeling immediately prior to electrofishing (day 0, predisturbance census) and then 1, 3, 10, 20, 30, and 40 days following the removal of target species. Control pools were snorkeled at 10-day intervals for 40 days. To minimize observer error, the same observer conducted surveys of all pools censused, and on each date, two consecutive censuses were performed at each control and experimental site. The mean of these two counts was used in statistical analyses. We evaluated the precision of this census technique by comparing snorkeling data with electrofishing data for the fish predisturbance census.

To confirm that riffles would not contribute a significant number of colonists to experimental pools, we electrofished six upstream riffles adjacent to treatment pools (three long-riffle and three short-riffle pools) on the final day of the recovery experiment. The results indicated that fish assemblages in riffles were very different from those in pools. Riffles contained only five of eight target species and lower numbers and smaller fish than pools (Table 3).

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Table 2. Mean (range) physical habitat and predisturbance assemblage characteristics of treatment and control pools.

	Pool characteristics				Riffle characteristics		Biological conditions	
	Length (m)	Width (m)	Area (m^2)	Depth (cm)	Length (m)	Depth (cm)	Density (fish/m^2)	Species richness
Riffle length								
Short	30 (15–59)	6.0 (4.9–7.1)	182 (74–418)	68 (50–100)	9.1 (2–20)	13.1 (7–18)	0.92 (0.6–1.5)	6.3 (5–8)
Long	30 (14–48)	6.5 (5.0–8.5)	202 (83–406)	60 (50–80)	46.4 (20–69)	12.8 (7–18)	1.18 (0.9–1.9)	6.2 (5–8)
Pool position								
Upstream	29 (14–50)	5.5 (4.9–6.1) *	155 (74–262) **	63 (50–85)	27.4 (2–60)	9.9 (6–12) **	0.95 (0.51–1.6)	6 (5–7)
Downstream	31 (18–59)	6.9 (5.7–8.5)	223 (104–418)	65 (50–100)	25.5 (2–69)	15.5 (13–18)	1.18 (0.7–1.7)	6.7 (6–8)
Control pools	30 (11–47)	5.0 (4.5–6.1)	159 (52–283)	67 (55–100)	21.6 (5–54)	16 (11–20)	1.52 (0.89–2.1)	5.8 (4–8)

Note: Significant within-treatment differences: $^*P < 0.05$, $^{**}P < 0.01$ (two-sample *t*-tests).

Table 3. Catch-per-unit-effort (CPUE) and size of target species in Long and Blaylock creeks.

	CPUE (fish/100 m^2)			Mean standard length (max. standard length) (mm)		
Species	Riffle		Pool	Riffle		Pool
Central stoneroller	1.2	*	9.0	57 (82)		60 (103)
Striped shiner	0.5	*	5.1	55 (108)	*	69 (104)
Smallmouth bass	0.1		0.4	50 (63)	*	96 (207)
Redfin shiner	<0.1	*	0.1			50 (64)
Bigeye shiner	<0.1		0.3	48 (48)		51 (52)
Creek chub	0.1	*	0.9	63 (95)	*	97 (155)
Northern studfish	<0.1	*	0.7	72 (85)		70 (95)
Longear sunfish	0.0	*	1.0			77 (116)
Total	1.90	*	17.3			

Note: Values represent means from six pools and six associated riffles in the two streams. Significant differences: $^*P < 0.05$ (two-sample *t*-test).

From these findings, we were confident that riffles did not provide a significant source of colonists to the experimental pools.

Statistics

Results from both streams were combined in all statistical analyses unless we detected significant differences in recovery between the two streams. We estimated pool-specific recovery rates (per day) for assemblages (species richness, percent similarity, total numbers), target species (numbers), or target size-classes (numbers) by linear regression analysis. Percent similarity was calculated using the percent similarity index (Wolda 1981). Because the absolute number of fish in control and experimental pools varied widely, we determined a relative number of fish for each pool by dividing counts (individuals per pool) obtained for any post-disturbance census by counts obtained on day 0. The model that best explained the relationship between time and recovery had the following linear form:

$$\ln(\text{Recovery}) = \ln(a) + b \ln(\text{day} + 1)$$

where ln(Recovery) represented the natural log of either assemblage (species richness, percent similarity, or relative numbers), species, or size-class recovery and b, the regression coefficient, represented the recovery rate ($\ln(\text{Recovery})/\ln(\text{day} + 1) - \ln(a)$). Although assemblage and species recovery rates generally declined towards an asymptote over time, we did not use an asymptotic regression model in these analyses. For our data, asymptotic regressions did not explain changes in recovery as well as logrithmic regressions. The same conclusion was reached in a similar study by Sheldon and Meffe (1995).

We used ANOVA and regression models to evaluate patterns of recovery between treatment groups and across pools. To test whether recovery differed between pools in each of the two treatment categories (riffle length and pool position), we compared mean recovery rates (b) for treatment categories using a two-way ANOVA. We used one-way ANOVA to compare differences in mean recovery rates among species and between size-classes. We determined the effects of other independent variables (i.e., riffle depth, distance to large pool, pool area, and predisturbance assemblage size) on pool-specific recovery rates by least squares regression analysis. Finally, we generated multiple regression models to describe the relative importance of each independent variable on assemblage recovery rates. Only uncorrelated variables were in-

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Table 4. Comparison of mean fish densities (individuals/m^2 ± 1 SE) estimated from electrofishing and snorkeling surveys of 12 treatment pools in Long and Blaylock creeks.

Species	Electrofishing	Snorkeling
Central stoneroller	0.61±0.23	0.62±0.22
Striped shiner	0.24±0.03	0.24±0.03
Longear sunfish	0.08±0.02	0.11±0.04
Creek chub	0.07±0.03	0.09±0.04
Northern studfish	0.03±0.01	0.06±0.03
Redfin shiner	0.03±0.01	0.03±0.01
Bigeye shiner	0.02±0.01	0.03±0.02
Smallmouth bass	0.02±0.01	0.02±0.01
Total density	1.10±0.26	1.21±0.29

cluded in this analysis. We performed log-transformations on data that deviated significantly from normality.

Results for control pools were examined by regression analysis to evaluate temporal variation in species richness, percent similarity, and assemblage and species relative numbers. Comparisons of results for control and experimental pools were made on days 0, 10, 20, 30, and 40 using two-sample *t*-tests.

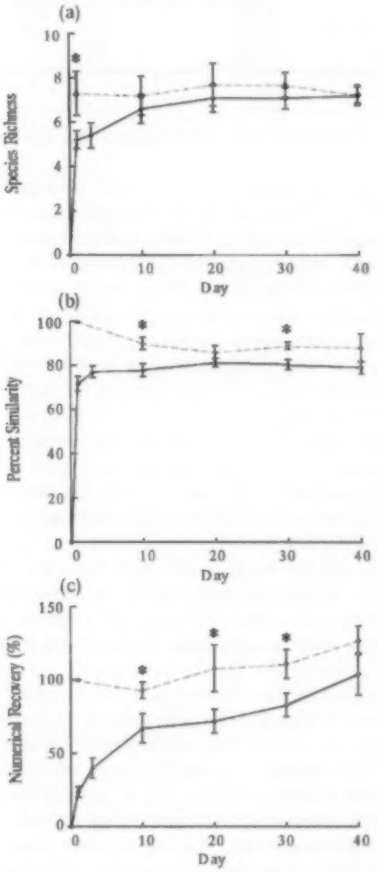
Results

Snorkeling surveys produced results that were comparable with those obtained in electrofishing surveys. Estimates of total fish per pool for the two methods were highly correlated (Pearson product-moment correlation coefficient, r = 0.95, n = 12, $P < 0.01$). Further, there was little difference between the two methods in the average densities of individual species and species rank (Table 4). Average assemblage similarity between the two techniques was 95%.

When measured in terms of species richness and percent similarity, the recovery of assemblages to predisturbance conditions occurred relatively rapidly and at similar rates in all 12 pools (Figs. 2*a* and 2*b*). Pool-specific recovery rates in species richness and overall percent similarity were not associated with any measured physical (riffle length, pool size, distance to large pool) or biological variable (initial assemblage size, $P > 0.20$). On average, the number of species per pool exceeded 50% of predisturbance values by day 10. By day 20, species richness stabilized, although at a value slightly lower than the predisturbance mean (Fig. 2*a*). With the exception of day 1, the mean number of species in experimental pools was not significantly different from means in control pools across all comparable dates ($P > 0.30$). Patterns of recovery based on percent similarity were similar to patterns described for species richness (Fig. 2*b*). Pool assemblages recovered to nearly 80% (range 49–89%) of their predisturbance composition by day 3 and fluctuated only slightly over the remainder of the recovery period. Species dominant in initial surveys (e.g., central stoneroller, striped shiner, creek chub) remained dominant during the recovery period. In control pools, percent similarity values (relative to initial assemblage structure) varied only slightly over the 40-day period (range 78–98%). Moreover, percent similarity values from control and experimental pools for comparable dates generally were very similar.

Species that returned most rapidly to experimental pools included the two most common and widespread species,

Fig. 2. Temporal trends for three assemblage characteristics (species richness, percent similarity, percent numerical recovery) in experimental (solid lines) and control pools (broken lines). Error bars represent ± 1 SE. Significant differences between control and experimental pools on comparable dates are indicated (*$P < 0.05$, two-sample *t*-tests).



central stoneroller (1.6 days) and striped shiner (1 day), but also two relatively uncommon species, bigeye shiner (2 days) and northern studfish (5.3 days). Redfin shiner was the slowest to recolonize the pools (19.2 days) and was also the least common species in the two streams.

In contrast with species richness and similarity, percent numerical recovery of assemblages occurred at a relatively slow rate (Fig. 2*c*). Typically, the relative number of fish increased rapidly during the earliest phases of the recolonization period, with some pools achieving 50% of their predisturbance numbers by day 3. Towards the conclusion of the recovery period, the number of fish per pool generally stabilized or increased very slowly. The number of fish in control pools varied more dramatically than values for species richness and percent similarity and appeared to increase slightly over the course of the study period (r^2 = 0.11, P = 0.09) (Fig. 2*c*). Unlike species richness and percent similarity, the mean number of fish in experimental pools was lower than the mean number of fish in control

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Table 5. Two-way ANOVA results showing effects of riffle length and pool position in the drainage on numerical recovery rates.

Source	Sum of squares	df	*F*-ratio	*P*-value
Length	0.009	1	26.7	<0.01
Position	0.004	1	12.4	<0.01
Position × Length	0.001	1	3.6	0.10
Error	0.002	8		

pools on all comparable dates except day 40 (two-sample *t*-test).

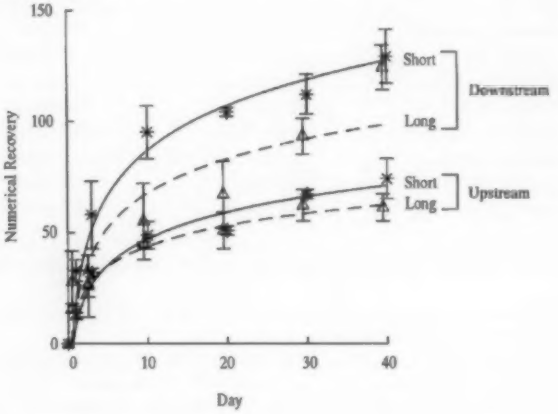
Numerical recovery was influenced strongly by factors that isolated experimental pools from potential colonists. We detected significant within-treatment differences in recovery rates for both riffle length and pool position ($P < 0.05$, two-way ANOVA) (Table 5). Recovery patterns were similar in the two streams, and significant relationships between time and recovery were found for all 12 experimental pools. For both upstream and downstream sites, numerical recovery was much more rapid in pools isolated by short riffles than by long riffles (Fig. 3). In short-riffle pools, assemblages reached full numerical recovery by day 30 whereas assemblages in long-riffle pools reached only 75% of their predisturbance densities by day 40. For the entire recovery period, the mean (± 1 SE) recovery rate (*b*) in large-riffle pools was 30% lower (0.13 ± 0.01) than the rate for short-riffle pools (0.19 ± 0.01). Consistent with the assemblage-level results, numerical recovery for individual species was more rapid in short-riffle pools (Table 6). Of the eight target species, two showed significant between-group differences in recovery over time.

Pool location in the drainage also had an effect on numerical recovery. For both long- and short-riffle pools, upstream assemblages recovered more slowly than those downstream (Fig. 3). On average, assemblages in downstream pools achieved full recovery by day 30 of the recolonization period whereas assemblages in upstream pools had not reached 70% recovery by day 40. The mean recovery rate (± 1 SE) for assemblages in upstream pools was 30% lower (0.13 ± 0.01) than downstream sites (0.18 ± 0.01). Three of seven species that occupied both upstream and downstream locations recovered to predisturbance numbers more rapidly downstream (Table 6).

The remaining factors that were associated with numerical recovery of assemblages were riffle depth and the distance to nearest large source pool. By contrast, neither predisturbance assemblage size nor pool area influenced numerical recovery rates of assemblages ($r^2 = 0.01$, $P > 0.50$). Riffle depth had a positive effect on numerical recovery rates ($r^2 = 0.37$, $P < 0.05$), although this relationship was confounded by pool position. We found no relationship between numerical recovery rate and riffle depth when we tested pools within each position category separately. Numerical recovery rates of assemblages were correlated negatively with the distance to large source pools ($r^2 = 0.44$, $P < 0.05$). This pattern held even when we tested pools within each riffle length or pool location category independently.

When we combined all independent variables in a multiple regression analysis, the resulting model included riffle length (Length), distance to large source pool (Distance), and riffle depth (Depth) as significant predictors of pool-specific numerical recovery rates ($P < 0.01$). The model $b = 0.21 - 0.01$ ln (Distance) $- 0.055$(Length) $+ 0.006$(Depth) explained nearly all of the variation in recovery rates among the 12 pools ($r^2 = 0.95$).

Fig. 3. Comparisons of percent numerical recovery for the riffle length and upstream and downstream treatment categories. Error bars represent ± 1 SE. Data points and error bars are offset to increase clarity.



Size-class and species comparisons

Broad differences were detected in the recovery rates of size-classes and different taxonomic groups (Fig. 4). Large fish recolonized experimental pools at a faster rate ($b = 0.19$) than small fish ($b = 0.13$) ($P < 0.05$, one-way ANOVA). For species included in this comparison (smallmouth bass, creek chub, and longear sunfish), large fish completely recovered to their predisturbance numbers by day 30. Small individuals had not even achieved 70% numerical recovery by the same date. Among the most common species in the two streams, three (smallmouth bass, central stoneroller, and longear sunfish) returned to predisturbance abundances by day 40 (Figs. 4*b* and 4*c*). By contrast, recovery rates for creek chub and striped shiner were much slower ($P < 0.01$, one-way ANOVA). For creek chub, numerical recovery was just slightly greater than 50% by day 40 (Fig. 4*c*).

Discussion

General trends in recovery

For general patterns of recovery across all 12 pools, we found rapid recovery (<40 days) in overall numbers, species richness, and percent similarity in experimental pools of Long and Blaylock creeks. Average values of all three measures of assemblage structure (species richness, percent similarity, and relative numbers) and relative numbers of most species and size-classes returned to predisturbance levels by the end of the 40-day sampling period. We attribute this high degree of resilience and predictable structure of assemblages to recolonization rates by species and size-classes in proportion to their abundance in neighboring habitats. However, it

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Table 6. Species regression coefficients (± 1 SE) showing recovery rates (*b*) for different treatments.

	Riffle length			Location in drainage		
Species	Long		Short	Upstream		Downstream
Central stoneroller	0.13	*	0.21	0.15	*	0.19
	0.03		0.03	0.04		0.02
Striped shiner	0.10	*	0.15	0.09	*	0.14
	0.02		0.01	0.02		0.02
Smallmouth bass	0.17		0.20	0.19		0.17
	0.01		0.02	0.05		0.01
Creek chub	0.10		0.08	0.11		0.08
	0.02		0.03	0.03		0.02
Longear sunfish	0.15		0.18	0.13	*	0.20
	0.02		0.03	0.02		0.02
Redfin shiner	0.27		0.25	0.19	$P < 0.10$	0.34
	0.03		0.08	0.03		0.08
Bigeye shiner	0.28		0.35			0.32
	0.01		0.08			0.04
Northern studfish	0.14		0.15	0.09	$P < 0.10$	0.19
	0.06		0.03	0.02		0.05

Note: Coefficients were derived using the following model: ln(Percent recovery) = ln(a) + $b$ln(day + 1). Significant differences: *$P < 0.05$ (one-way ANOVA).

should be noted that this type of proportional colonization was not always evident for individual species (i.e., creek chub and striped shiner).

General trends in assemblage and species recovery described in this study were similar to those of two recent studies that investigated fish recolonization at the scale of habitats and short stream reaches (e.g., Peterson and Bayley 1993; Sheldon and Meffe 1995). Peterson and Bayley (1993) recorded assemblage recovery in <10 days in stream reaches ranging from 46 to 113 m and Sheldon and Meffe (1995) within 23 days in short pools (10–23 m in length). Moreover, results from all three studies indicate that species richness and percent similarity recover to predisturbance levels more rapidly than total numbers of fish.

Agreement in the findings of these studies reinforces the view of Sheldon and Meffe (1995) that stream fishes can be highly mobile and that spatially restricted episodic disturbances may produce only short-term impacts on fish assemblages. However, as Sheldon and Meffe (1995) cautioned, impacts can become severe under conditions of chronic disturbance or for populations of rare species or locally adapted genomes.

Most species recovered to their predisturbance numbers at similar rates, although two very common cyprinids (creek chub and striped shiner) colonized pools at rates about 50% lower than other species. These results indicate that recolonization rates for individual species cannot be predicted solely by knowing their proportions in neighboring habitats. Other factors, such as differences in home range size, also may influence species recovery patterns.

In addition to species-specific differences in recolonization, our results indicated that large fish (>100 mm) recolonized pools more rapidly than small fish. Little has been published on size-specific patterns of recovery (Detenbeck et al. 1992), and the literature on movement by stream fish has yielded mixed results on the mobility of different size-classes. For example, Bachman (1984) suggested that a negative correlation between home range size and fish length in brown trout (*Salmo trutta*) was caused by large fish outcompeting small fish for optimal foraging locations. Alternatively, it also has been shown that home range size increases with fish size (Allen 1951; Gerking 1953; reviewed by Minns 1995). A possible explanation for this relationship is that large fish have higher energy demands and require larger foraging areas (Gerking 1953). Of the species surveyed in this study, longear sunfish are known to exhibit size-specific movements consistent with the second view (Gerking 1953; Gunning and Shoop 1963).

Effects of physical factors on recovery

Physical factors can influence fish recolonization (reviewed by Detenbeck et al. 1992). However, most studies that have examined these influences have been carried out at relatively large spatial scales (i.e., reaches, streams) and focused on large-scale stream attributes such as latitude, gradient, stream order, watershed size, barriers to migration, and distance to source populations. Of these factors, only those that directly affected rates of movement (i.e., barriers to migration and distance to source populations) were correlated with recovery patterns (Detenbeck et al. 1992). Our study has shown that factors affecting rates of movement are also important determinants of fish recolonization when this process is measured at the scale of habitat units. All four measures of pool isolation (riffle length, riffle depth, position in drainage, and distance to large pools) affected numerical recovery. The only measured variables not linked to movement (pool size and predisturbance assemblage size) also had no apparent influence on rates of numerical recovery.

Generally, the least isolated pools (downstream, short riffles) achieved full recovery by day 20 whereas estimates for the most isolated pools (upstream, long riffles) were typically much greater than 40 days. The length of time necessary for these pools to achieve full recovery will be influenced by various factors, including seasonal changes in stream flow, fish behavior, and abundance.

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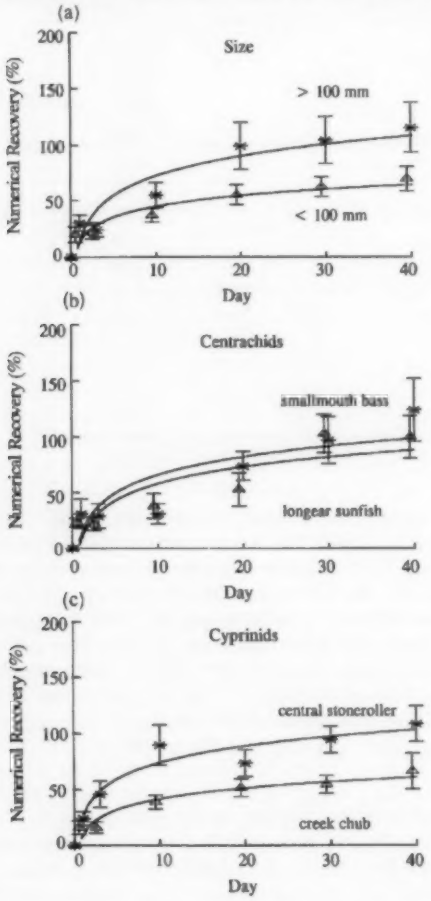
Fig. 4. Percent numerical recovery for different species and size-classes across all 12 experimental pools. Error bars represent ± 1 SE. Data points and error bars are offset to increase clarity.



The influences of isolation on numerical recovery of stream habitats have been discussed to varying degrees in previous studies. Peterson and Bayley (1993) suggested that riffle depth may have lengthened the recovery time of stream assemblages in a Missouri river. Although their conclusion was based on circumstantial evidence from one riffle, it is supported by the positive relationship between riffle depth and assemblage recovery rates detected here. The role of isolation on fish recolonization also was discussed by Sheldon and Meffe (1995) who described differences in assemblage-level recolonization rates of upstream and downstream pools. They speculated that the distance from source areas (i.e., neighboring streams) could have been responsible for this pattern. However, it was acknowledged that both upstream and downstream sites contained undisturbed habitats that could have provided significant numbers of colonists to the defaunated pools and that physical gradients may have been important. We also suspect that differences in recovery rates between upstream and downstream pools in our study were at least partly influenced by habitat differences. For example, upstream riffles were shallower than those downstream (10 versus 15 cm) and recovery was associated with riffle depth.

One other significant finding of our study was that numerical recovery rates declined as the distance of experimental pools to large source pools increased. Sedell et al. (1990) asserted that large pools are very important to the ecology of streams because they provide stream organisms with refugia during stressful conditions (e.g., droughts, floods). Our results imply that large pools, which in these two streams were two times larger than average-sized pools, also may serve as important sources of immigrants to neighboring upstream and downstream habitats.

In the present study, experimental pools were isolated from neighboring pools by riffles ranging from <10 to slightly more than 50 m in length. Over this range, numerical recovery varied greatly. Mean recovery rates were 33% slower in the isolated pools, and time to full recovery ranged from <20 days in short-riffle pools to much greater than 40 days in long-riffle pools. While our data indicate that isolation can influence fish recolonization of habitats following disturbances, there may be other ecologically important consequences of this isolation. For example, routine movements of fish between habitats may depend to some extent on the spacing of habitat units. In fact, this was suggested by Gerking (1953) in a study of fish movement. Gerking (1953) found that only a very small percentage of tagged individuals representing several different species moved between two pools separated by a long riffle.

The importance of habitat isolation to the ecology of stream fishes may vary widely across streams and geographic regions in association with physical factors and land-use activities that determine channel characteristics. Riffle lengths in our streams were probably representative of conditions in many small eastern upland stream systems, but they were much shorter than those found in small montane streams of the Pacific Northwest (e.g., >80 m, Beechie and Sibley 1997). In contrast, low-gradient, sandy-bottom Gulf and Atlantic Coastal Plain streams may lack physical conditions promoting riffle development. Logging, agriculture, and other land-use activities carried out along streams also can lead to changes in the channel course or reduce the availability of in-stream elements (e.g., large woody debris) that create habitat (Karr and Schlosser 1978). Such changes reduce the abundance, and therefore the spacing, of pools in streams. Although numerous studies have described significant impacts of land-use disturbances on stream fishes, they generally have been focused at the scale of individual habitat units. We found that assemblage recovery, measured within habitats, was related to reach-scale characteristics (i.e., riffle depth, length, distance to large source pools) that affect habitat isolation. These results have potentially important implications for research aimed at understanding the ecology of stream fishes and predicting the consequences of land-use activities.

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Effects of different organic carbon sources on a freshwater plankton community[1]

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Abstract: We examined effects of terrestrial and aquatic detritus on optical, chemical, and biological properties of a planktonic food web in mesotrophic Calder Lake, New York. The 5400-L mesocosms received detritus from terrestrial leaves or aquatic macrophytes (added 1–2 mg dissolved organic C/L). Terrestrial detritus had significant effects on UV-B absorbance (OD_{250} twofold greater), but humic substances (OD_{440}) remained low. Both C sources resulted in 40% declines in oxygen saturation and two- to threefold increases in dissolved inorganic and total P but no effects on NH_4^+ or NO_3^-. Bacterial growth was stimulated by 20% in systems receiving macrophyte C but declined by 50% in systems with terrestrial C. Initially, controls had >60% greater chlorophyll *a* concentrations than C-amended systems, but mesocosms with macrophyte C exhibited the greatest chlorophyll *a* concentrations after day 10. Autotrophic nanoflagellates <20 μm (*Chrysochromulina parva*, *Ochromonas*) increased in controls but were inhibited in C-amended systems. Cyanobacteria (*Coelosphaerium naeglianum*, *Anabaena*, *Dactylococcopsis acicularis*) numbers increased in mesocosms with macrophyte C; densities of most algal taxa declined in terrestrial-supplemented systems, except certain flagellates (*Synura*, *Cryptomonas*, *Ceratium hirundinella*), which were enhanced. Abundances of heterotrophic nanoflagellates, rotifers, *Daphnia pulicaria*, and copepod nauplii were stimulated by macrophyte C; only the copepod *Mesocyclops edax* increased following addition of terrestrial C.

Résumé : Nous avons examiné les effets de détritus terrestres et aquatiques sur les propriétés optiques, chimiques et biologiques d'un réseau trophique planctonique dans le lac mésotrophe Calder (État de New York). Des mésocosmes de 5 400 L ont reçu des détritus de feuilles de plantes terrestres ou de macrophytes aquatiques [avec addition de 1–2 mg de carbone organique dissous (COD) par litre]. Les détritus terrestres avaient des effets significatifs sur l'absorbance des UV-B (doublement à DO_{250}), mais les teneurs en substances humiques (DO_{440}) restaient faibles. Pour ces deux sources de C, on a observé des diminutions de 40 % de la saturation en oxygène, des augmentations par un facteur 2 à 3 des composés inorganiques dissous et du P total, mais aucun effet sur les concentrations de NH_4^+ ou de NO_3^-. La croissance bactérienne était stimulée par un facteur de 20 % dans les systèmes recevant du C de macrophytes, mais elle diminuait de 50 % dans ceux qui recevaient du C de détritus terrestres. Au début, les témoins présentaient des augmentations des concentrations de Chl-*a* supérieures de plus de 60 % par rapport aux systèmes modifiés par l'ajout de C, mais les mésocosmes ayant reçu du C de macrophytes présentaient les plus fortes concentrations de Chl-*a* après le 10^e jour. Les populations de flagellés autotrophes de moins de 20 μm (*Chrysochromulina parva*, *Ochromonas*) augmentaient chez les témoins, mais elles étaient inhibées dans les systèmes modifiés par l'ajout de C. Les populations de cyanobactéries (*Coelosphaerium naeglianum*, *Anabaena*, *Dactylococcopsis acicularis*) augmentaient dans les mésocosmes enrichis en C de macrophytes, et les densités de la plupart des taxons d'algues diminuaient dans les systèmes enrichis en C de détritus terrestres, sauf celles de certains flagellés (*Synura*, *Cryptomonas*, *Ceratium hirundinella*), chez lesquels on notait une augmentation. Les abondances des nanoflagellés hétérotrophes, des rotifères, des *Daphnia pulicaria* et des nauplius de copépodes étaient rehaussées par l'apport de C de macrophytes; seul le copépode *Mosocyclops edax* profitait de l'ajout de C de détritus terrestres.

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Introduction

A central goal in microbial ecology is to characterize the nature of coupling between dissolved organic matter (DOM) and aquatic organisms. Higher quality, phytoplankton-produced DOM is regarded as the most important C source for bacteria, but aquatic macrophytes and terrestrial plant matter may provide the majority of the DOM pool in many small lakes (Wetzel et al. 1995). As most lake ecosystems are small, the influence of littoral and terrestrial inputs can be of considerable importance.

Generally, bacterioplankton production rates correspond well with measured phytoplankton production or dissolved

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organic C (DOC) across systems (Cole et al. 1988). However, at times, DOC concentration correlates poorly or inversely with either bacterial numbers or activity at specific sites (Findlay et al. 1991; Le et al. 1994; Robarts et al. 1994). Such results are likely due in part to variations in the quality of DOC supplied to the ecosystem, as well as activities of other members within the pelagic food web. For example, phytoplankton-derived DOC has been shown to support substantial production and high growth efficiencies in bacterioplankton in a variety of ecosystems (Sundh 1989; Kirchman et al. 1991). In general, however, extracellular release of DOM by phytoplankton is thought to be insufficient to meet bacterial demand in some systems (Baines and Pace 1991). Submersed and emergent aquatic macrophytes may also be a major source of DOC for planktonic microbial communities. Phagotrophic protists may enhance mineralization of macrophyte DOM, although this source of DOM may have no significant effects on protist biomass (Johnson and Ward 1997). The importance of aquatic macrophytes therefore must be evaluated from these points of view as well as the extent of the littoral zone or adjacent wetland.

The C budgets of small lakes are also influenced by inputs of organic matter from terrestrial sources, which are often the primary source of DOC in humic lakes (Tranvik 1992). For most lakes in deciduous forests, allochthonous particulate organic C (POC) enters lakes during autumn, with gradual leaching and decomposition of recalcitrant DOC by bacteria over the year (Wetzel 1995; Arvola et al. 1996). Small lakes, because of their size relative to the forest–watershed area, may be influenced by terrestrial DOM to a greater degree. It has been regarded as a paradox whether this large, mostly recalcitrant DOC pool supports continuous, slow growth of bacterioplankton or in fact retards microbial growth (Tranvik 1992). The fate and stimulatory effects of terrestrial leaves in lotic environments are well documented (e.g., Kaplan and Newbold 1993), and growth of stream bacteria is affected by both the source of DOM and its concentration (Koetsier et al. 1997).

Less is known about the effects of terrestrial C on planktonic communities, despite their probable importance in many small lakes. The effects of allochthonous C on pelagic food webs are likely to be both direct and indirect and may be complex. For example, addition of terrestrial humic material was found initially to stimulate, but later inhibit, phytoplankton primary production in an impounded lake, perhaps due to short-term release of P and gradually reducing light penetration (Guildford et al. 1987). Effects of humic materials in one small boreal lake were season dependent, with stimulation of bacterioplankton and reduction of phytoplankton during summer and autumn but not in spring (Arvola et al. 1996).

Our goal was to quantify the effects of detritus from two sources: aquatic macrophytes and terrestrial leaves. Experiments tested these effects in a small lake that is not colored or humic influenced. We hypothesized that (*i*) both macrophyte and terrestrial (leaf) organic C will stimulate bacterioplankton, but macrophyte C will have a greater effect, (*ii*) phytoplankton biomass will be enhanced by macrophyte C and reduced by terrestrial C, (*iii*) both DOM enrichments will enhance protist numbers following increases in bacterioplankton, and (*iv*) numbers of larger zooplankton will decline following terrestrial C treatments and increase with macrophyte C.

Methods

Study site

Experiments were conducted at the experimental lakes facility of the Louis Calder Center (Biological Station of Fordham University) in Armonk, N.Y. The facility receives whole lake water from Calder Lake, a shallow, mesoeutrophic system ($\bar{Z}$ = 2.8 m, Z_{max} = 6.7 m) with a substantial submersed macrophyte community (*Vallisneria americana*, *Elodea canadensis*, *Potamogeton amplifolius*, *Najas flexilis*) covering more than 50% of the lake bottom. In late summer, macrophyte detritus begins to decay throughout the lake. Because of its small size (about 4 ha), the lake also receives a proportionately large subsidy of forest leaves (*Quercus rubra*, *Quercus prinus*, *Acer rubrum*, *Fagus grandifolia*, *Cornus florida*) in the autumn. Prior studies have demonstrated that production rates of bacteria and larger phytoplankton in Calder Lake are primarily limited by the supply of inorganic P (Wehr 1989; Toolan et al. 1991; Le et al. 1994). Annual variations in concentrations of DOC in Calder Lake vary between 4 and 6 mg C/L (annual coefficient of variation about 20%).

Experimental design

Experiments were run in twelve 5400-L fiberglass mesocosms (1.9 m in diameter × 2.1 m deep) located about 100 m from Calder Lake in a large clearing at the edge of a forest. Enclosures were scrubbed and rinsed with (spring-fed) well water and then rinsed with lake water (pumped from 1 m depth) prior to filling. Whole lake water (plus bacteria, phytoplankton, zooplankton) was pumped into 12 tanks during the night (15 July 1996) to ensure a representative sample of larger cladocerans, which migrate vertically. Experimental POC treatments were employed to approximate a late summer – autumn import of POC from either terrestrial leaves or decaying aquatic macrophytes. Mesocosms were open to sunlight about 12 h a day (21 out of 22 days full sun or partial clouds), but covered with coarse-mesh nylon screening (about 8-mm mesh) to prevent leaves from entering the tanks. It was the intent of the present study to compare effects of different sources of C rather than a concentration effect. Field measurements in recent years indicate short-term increases in DOC during autumn of 1–2 mg C/L. The amount of POC required to amend mesocosm DOC by 2 mg C/L was determined by laboratory pilot studies with each detritus source. Macrophytes were collected near the end of the growing season (July), with proportions of plant species used based on vegetation surveys in 1996. Freshly fallen terrestrial leaves were collected from the forest floor within 100 m of Calder Lake; proportions of tree species were based on a visual survey of trees clearly visible around the margin of the lake. Both sources were dried to constant weight (105°C) and ground in a Wiley mill (sieve size 0.5 mm) prior to use to simulate the initial shredding activity after plant detritus enters a lake. The three treatments (times four replicates) employed were (*i*) leaves: 15 mg POC/L from forest tree leaves (65% *Quercus*, 25% *Acer*, 10% *Fagus* plus *Cornus*), (*ii*) macrophytes: 18.5 mg POC/L from aquatic macrophytes (50% *Vallisneria*, 40% *Elodea*, 10% *Potamogeton*), and (*iii*) control: no POC addition. POC was added to each mesocosm while being filled with lake water to ensure complete mixing at time zero. Tanks were mixed manually every 5 days using a clean PVC pipe.

Sampling

Sunlight (photosynthetically active radiation (PAR), microein-

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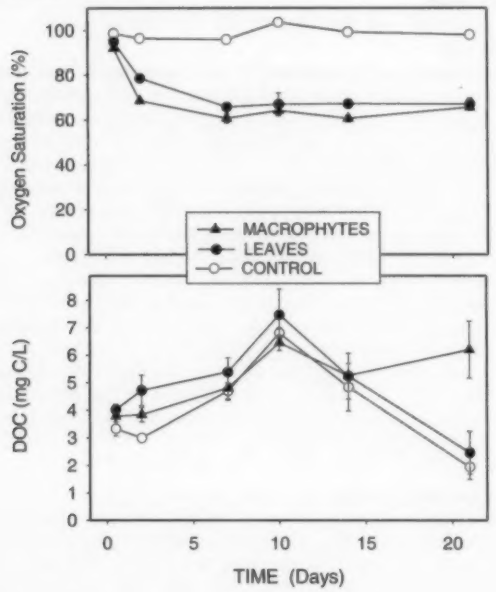
Fig. 1. Changes in surface O_2 percent saturation and DOC over time in Calder Lake mesocosms (at 0.5 m depth) amended with different detrital C sources (macrophyte detritus added, terrestrial detritus (leaves) added); means ± 1 SE, n = 4; errors less than symbol size not shown).



steins per square metre per second) was measured continuously and compared with weekly in situ depth profiles of underwater light climate in the mesocosms using a submersible (cosine corrected) PAR probe (Li-Cor instruments, model 185A). Most water samples were collected over 3 weeks at 0.5, 2, 7, 10, 14, and 21 days using a peristaltic pump at about 0.5 m depth and stored cold (about 4°C) until processed in the laboratory (<4 h). Phytoplankton, bacterioplankton, and flagellate samples were collected (20 mL each) unfiltered (days 0.5, 7, and 14). Algal samples were preserved with Lugol's iodine, bacteria were stored cold (about 4°C) unpreserved, and heterotrophic flagellates were preserved using cold (about 4°C) buffered glutaraldehyde. An additional unfiltered sample was collected for later measurement of UV absorbance. Samples for chlorophyll *a* (Chl *a*) measurements were collected (about 1000 mL) using a 47-mm Nalgene filter unit fitted with a Whatman (GF/F, about 0.7 μm) glassfiber filter. Water filtered through these filters (8 mL) was collected in acid-washed vials, acidified to pH <2.0 with trace element grade H_2SO_4, and stored cold until later water chemistry analyses. Dissolved O_2 levels and temperature were measured in situ using a YSI (model 57) O_2/temperture meter. Zooplankton samples were collected using a 12-L Schindler trap (days 0.5, 7, and 14) and preserved with formaldehyde to a final concentration of 2%.

Laboratory analyses

Water samples were filtered using a 0.2-μm pore size filter (polycarbonate, prerinsed with sample) immediately prior to measuring UV absorbance (within 2 h after sampling). Samples were measured at 250, 365, and 440 nm using a Shimadzu UV-160 spectrophotometer with matched quartz cuvettes (1-cm path length) versus deionized (Milli-Q plus) water as a reference. Absorbance measurements at 440 nm were used as index of humic materials (Kirk 1983), while values at 250 and 365 nm were used to quantify the relative amounts of small versus larger organic compounds, respectively, in DOC (Lindell et al. 1995). The ratio of OD_{250} to OD_{365} is greater if DOC contains proportionately more low molecular weight molecules (De Hann and De Boer 1987). Water chemistry samples were analyzed for soluble reactive P (SRP) using antimony–ascorbate–molybdate (American Public Health Association 1985; Bran+Luebbe Analyzing Technologies 1986*a*), NH_4^+ with phenol–hypochlorite, and NO_3^- (after reduction to NO_2^- in a Cd–Cu column) via reaction with sulfanilamide–NNED (American Public Health Association 1985; Bran+Luebbe Analyzing Technologies 1986*b*, 1987). Total dissolved P (TDP) was measured in an identical manner to SRP, following an acid–persulfate digestion. DOC was measured (after dissolved inorganic C removal via acid sparging) following digestion with acid–persulfate and high-energy UV; the CO_2 produced was dialyzed and reacted with buffered phenolphthalein and measured at 550 nm (Goulden and Brooksbank 1975; Bran+Luebbe Analyzing Technologies 1989). The above procedures were modified for automated analysis and run on a TrAAcs 800 automated analyzer (Bran+Luebbe Inc., Buffalo Grove, Ill).

Subsamples (10 mL) of Lugol's-preserved phytoplankton samples were placed in sedimentation chambers and allowed to settle for a minimum of 12 h. Identification and counting procedures followed the standard inverted microscope method (Utermöhl 1958) using a Nikon Diaphot microscope. A minimum of 10 grids and 1000 cells were enumerated from each sample. Bacterioplankton samples were preserved immediately prior (<4 h after sampling) to filtering (1 mL of sample plus 9 mL of particle-free water) with glutaraldehyde (to 2%), stained with DAPI, and filtered onto Irgalan black stained 25-mm polycarbonate filters (0.2-μm pore size, Poretics Corp.) (Porter and Feig 1980). Samples were stored frozen (–15°C) until later observation; counts were made at 1250× magnification using a Nikon Labophot epifluorescence microscope with a UV-2A filter set (excitation 330 nm, barrier 420 nm). A minimum of 10 grids and 300 cells were counted per sample replicate. Cell lengths and widths were measured to separate small (<0.4 μm) and large (> 0.4 μm) cells. Heterotrophic nanoflagellates (no autofluorescence with blue excitation) were prepared in a similar fashion, but 5-mL samples (plus 5 mL of distilled water) and 0.4-μm pore size filters were employed. Chl *a* samples were extracted cold (<4°C) in the dark with 90% neutral acetone for 12–24 h and then centrifuged and analyzed spectrophotometrically and corrected for pheophytin *a* (Lorenzen 1967; American Public Health Association 1985). Zooplankton samples were counted using a dissection microscope.

Data analyses

Analysis of variance (ANOVA) was used to test whether the POC sources had significant effects on biological and chemical variables measured; ANOVA tests were run datewise (days 2, 7, 10, and 14) to consider treatment and temporal differences in community responses. Prior to analyses, data were checked for assumptions of normality and homogeneity of variances. Nearly all variables (except absorbance at 250, 365, and 440 nm) were nearly normally distributed (based on skewness, kurtosis, probability plots); no transformations were employed. Individual comparisons among each treatment were made using Tukey's honestly significant difference (HSD) on a post hoc basis (Sokal and Rohlf 1995). For both ANOVA and HSD, effects or comparisons were judged to be significant if the probability (P) of a type I error (α) was <0.05. Data were complied and analyzed using SYSTAT v. 7.0 for Windows (Wilkinson 1997).

Results

O_2 saturation and DOC

The addition of both organic C sources had pronounced

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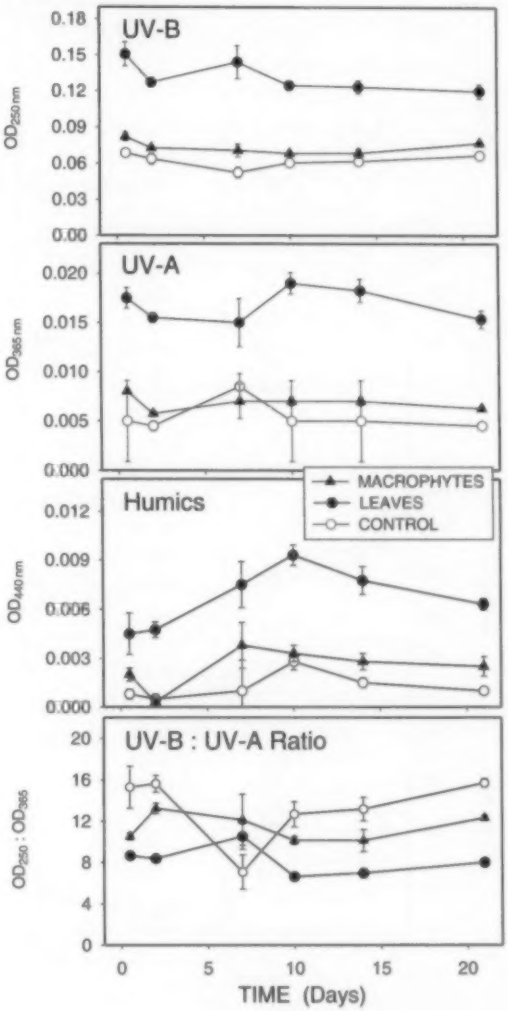
Table 1. Effects of POC treatments on physical and chemical variables in Calder Lake mesocosms measured on days 2–21.

Day	Variable	*F* score	*P*	Individual effects
2	O_2 saturation	802.88	<0.001	C > L > M
7		185.30	<0.001	C > L ≈ M
10		49.02	<0.001	C > L ≈ M
14		250.41	<0.001	C > L ≈ M
21		141.46	<0.001	C > L ≈ M
2	DOC	0.17	>0.5	L ≈ M ≈ C
7		0.10	>0.5	L ≈ M ≈ C
10		0.12	>0.5	L ≈ C ≈ M
14		0.05	>0.5	M ≈ L ≈ C
21		15.57	0.002	M > L ≈ C
2	Absorbance UV-B_{250}	90.00	<0.001	L > M ≈ C
7		30.28	<0.001	L > M ≈ C
10		190.46	<0.001	L > M ≈ C
14		77.82	<0.001	L > M ≈ C
21		86.02	<0.001	L > M ≈ C
2	Absorbance UV-A_{365}	193.44	<0.001	L > M ≈ C
7		5.05	0.034	L > C ≈ M
10		114.67	<0.001	L > M ≈ C
14		88.52	<0.001	L > M ≈ C
21		138.70	<0.001	L > M ≈ C
2	Humics A_{440}	51.17	<0.001	L > C ≈ M
7		1.39	0.30	L ≈ C ≈ M
10		45.95	<0.001	L > M ≈ C
14		31.50	<0.001	L > M ≈ C
21		120.80	<0.001	L > M > C
2	SRP	4.76	0.039	M ≈ L > C
7		17.46	0.001	M ≈ L > C
10		4.74	0.039	M ≈ L > C
14		7.31	0.013	L ≈ M > C
21		4.48	0.044	L ≈ M > C
2	TDP	2.52	0.136	M ≈ L ≈ C
7		11.90	0.003	M ≈ L > C
10		6.48	0.018	M ≈ L > C
14		3.08	0.096	L ≈ M ≈ C
21		0.48	>0.5	M ≈ L ≈ C
2	NH_4^+	0.09	>0.5	L ≈ M ≈ C
7		0.75	>0.5	C ≈ L ≈ M
10		2.41	0.14	M ≈ C ≈ L
14		2.70	0.12	M ≈ C ≈ L
21		1.30	0.31	M ≈ C ≈ L
2	NO_3^-	0.34	>0.5	M ≈ L ≈ C
7		1.65	0.25	M ≈ L ≈ C
10		1.06	0.39	M ≈ C ≈ L
14		0.65	>0.5	L ≈ M ≈ C
21		1.01	0.40	M ≈ C ≈ L

Note: $n = 4$ for each day; *F* score based on ANOVA; *P* = probability. Individual effects listed in order of magnitude; post hoc significance of contrasts based on Tukey's HSD (difference at $P < 0.05$) comparisons. C, controls; M, macrophytes; L, terrestrial leaves.

Fig. 2. Changes in the absorbance of filtered lake water from Calder Lake mesocosms amended with different detrital C sources (macrophyte detritus added, terrestrial detritus (leaves) added); means ± 1 SE.



effects on the O_2 balance in the mesocosms (Fig. 1). There were no significant temperature differences among treatments during the entire study. After 2 days, O_2 saturation had declined by 20–30% in systems receiving terrestrial and macrophyte detritus, respectively. By 7 days, O_2 consumption in all organic C amended systems resulted in fairly stable O_2 saturation levels of about 60%, while controls were at or near full saturation throughout the study (differences significant at all times via ANOVA; Table 1). Terrestrial and aquatic C sources had similar effects, although early in the study (day 2) the O_2 decline was significantly more rapid in systems receiving macrophyte than terrestrial detritus (Tukey's HSD, $P < 0.05$; Table 1). Carbon sources were added at levels designed to achieve target DOC increases of 1–2 mg C/L. This was nearly achieved in the terrestrial C treatments (Fig. 1), which averaged about 24% greater than

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Fig. 3. Concentrations of SRP, TDP, NH_4^+-N, and NO_3^--N in Calder Lake mesocosms amended with different C sources (macrophyte detritus added, terrestrial detritus (leaves) added); means ± 1 SE.

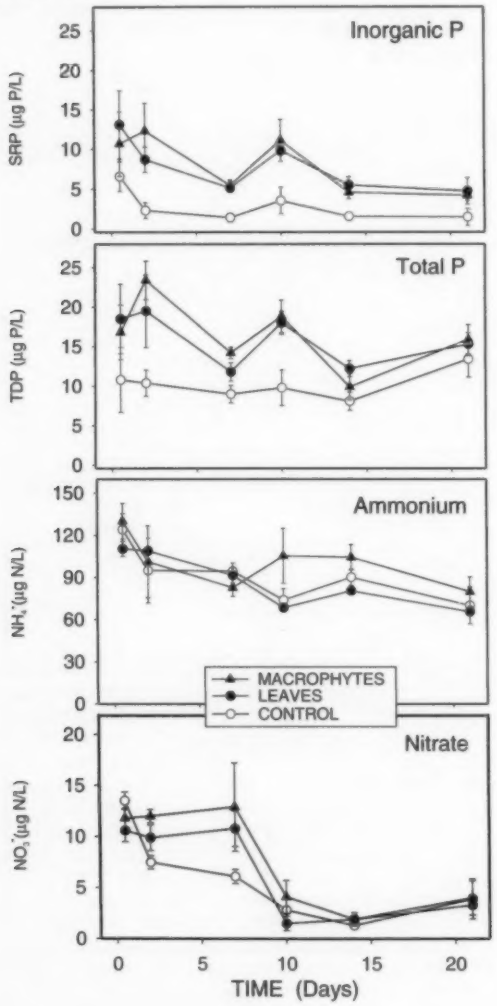
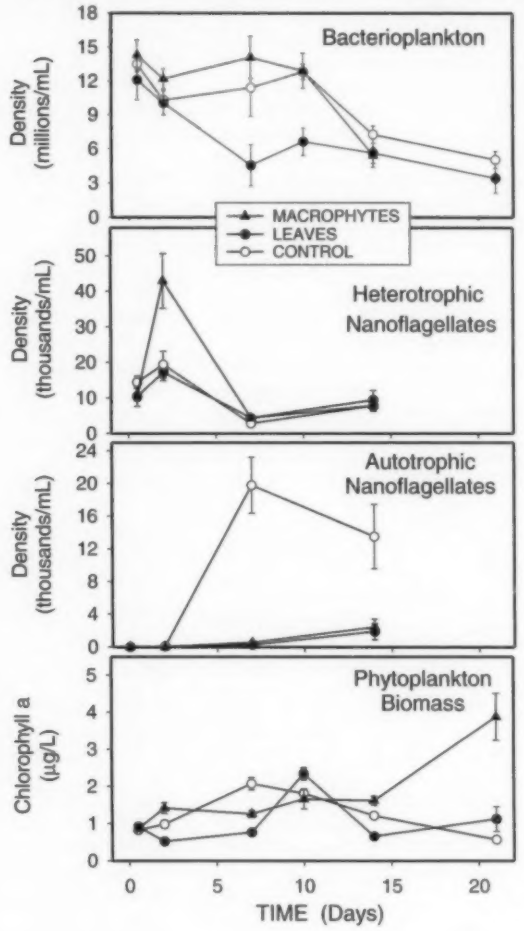


Fig. 4. Densities of bacterioplankton and heterotrophic and autotrophic nanoflagellates, and Chl *a* concentration in Calder Lake mesocosms amended with different C sources (macrophyte detritus added, terrestrial detritus (leaves) added); means ± 1 SE.



controls. Between days 0.5 and 14, DOC levels in macrophyte-amended systems averaged 9.5% greater than controls, but on day 21 the DOC concentration rose to more than twice that measured in controls. Despite strong effects on O_2 levels, a statistically significant increase in DOC was measured only on day 21 in mesocosms supplied with macrophyte detritus (Table 1). A gradual rise in DOC during the experiment from day 2 to day 10 (mean 6.7 ± 0.32 (SE)) was also observed in control mesocosms. A small pulse in DOC concentration was recorded in macrophyte-amended systems on day 21 (5.3–6.2 mg/L).

UV and light absorbance

Carbon treatments had distinct effects on light absorption characteristics (Fig. 2). Water appeared pale brown within 0.5 day but changed little over time. Mesocosms receiving terrestrial leaf detritus had 2.1-fold greater OD_{250} and 3.3-fold greater OD_{365} values than controls and macrophyte-amended systems ($P < 0.05$; Table 1). Macrophyte detritus had less of an effect on optical properties (10–20% greater; all comparisons $P > 0.10$). OD_{440} (an index of humic substances) was low in all systems, although measured values were significantly greater (about fivefold) in mesocosms amended with terrestrial leaf detritus than controls (Tukey's HSD, $P < 0.010$ on four out of five dates). Light reaching mesocosms was fairly high during the 3-week period and averaged 40 400 $\mu E \cdot m^{-2} \cdot day^{-1}$ (about 468 $\mu E \cdot m^{-2} \cdot s^{-1}$). Subsurface irradiance extinction patterns on day 14 indicated that about 30% of surface PAR was recorded at 1 m depth in systems receiving terrestrial POC, about 58% surface PAR in macrophyte-amended systems, and 68% in controls. Log(percent transmission) plots versus depth indicted that the slopes of the three extinction rates were each significantly different from each other (*t*-tests, $P < 0.005$ for each).

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Table 2. Effects of POC treatments on bacterioplankton, flagellates, and phytoplankton in Calder Lake mesocosms measured on days 2–14.

Day	Variable	*F* score	*P*	Individual effects
2	Bacterioplankton	2.03	0.187	M ≈ C ≈ L
7		5.13	0.029	M ≈ C > L
10		8.08	0.012	M ≈ C > L
14		1.18	0.351	C ≈ L ≈ M
21		1.19	0.349	C ≈ M > L
2	Autotrophic nanoflagellates	0.50	>0.5	L ≈ M ≈ C
7		31.70	<0.001	L ≈ M < C
10		nd		
14		7.45	0.012	L ≈ M < C
2	Phytoplankton Chl *a*	18.69	<0.001	M > C > L
7		31.16	<0.001	C > M > L
10		3.61	0.071	L ≈ M ≈ C
14		28.32	<0.001	M > C > L
21		18.29	0.001	M > L ≈ C
2	Heterotrophic nanoflagellates	8.21	0.009	M > C ≈ L
7		1.37	0.266	L ≈ M ≈ C
10		nd		
14		0.32	>0.5	L ≈ M ≈ C
2	Cyanobacteria <2 μm	0.129	>0.5	L ≈ C ≈ M
7		2.123	0.176	C ≈ M ≈ L
10		32.110	<0.001	C > M > L
14		5.281	0.030	C > M ≈ L
2	Colonial cyanobacteria	0.991	0.408	L ≈ C ≈ M
7		2.750	0.117	M ≈ C ≈ L
10		1.170	0.354	M ≈ L ≈ C
14		13.214	0.002	M > C ≈ L
2	Chlorophytes	0.217	>0.5	C ≈ L ≈ M
7		3.257	0.086	M ≈ C ≈ L
10		1.111	0.371	M ≈ C ≈ L
14		1.409	0.294	L ≈ C ≈ M
2	Chrysophytes plus dinoflagellates	8.247	0.009	M ≈ C > L
7		0.274	>0.5	C ≈ L ≈ M
10		6.144	0.021	L > M ≈ C
14		2.500	0.137	L ≈ M ≈ C

Note: $n = 4$ for each day; day 21 sampled for bacteria; nd, no data. See Table 1 for further details.

P and N effects

Additions of allochthonous C had significant effects on P levels throughout the study (Fig. 3). Within 0.5 day, inorganic P (SRP) levels in both organic C amended systems were more than twice (terrestrial C: 3.5 times; macrophyte C: 3.3 times) the levels measured in controls (significant all times; Tukey's HSD, $P < 0.05$; Table 1). All systems regardless of treatment experienced a decline in SRP over time, although detritus treatments apparently maintained SRP levels above 5 μg/L. No significant differences in SRP were observed between the systems receiving different C sources. TDP levels were also elevated (macrophyte and terrestrial C: 2.0 times), but effects were judged significant only on days 7 and 10 (Fig. 3; Table 1). During the summer of 1996, NH_4^+ was the predominant N ion in Calder Lake (>90% total dissoved inorganic N). Detritus additions failed to substantially change the N economy of any systems or prevent the rapid declines in NO_3^- that were observed in control systems. Slight elevations in NH_4^+ were observed in systems receiving macrophyte C after day 7, but in general, neither detrital treatment had a large or statistically significant effect on either NH_4^+ or NO_3^- in the water column (Table 1).

Effects on bacterioplankton, flagellates, and phytoplankton

Bacterial numbers were strongly affected by organic C treatments (Fig. 4). Macrophyte C apparently stimulated bacterial growth by midexperiment (about 20% increase), while terrestrial C addition resulted in 40–60% declines in bacterioplankton numbers. These inhibitory effects were significant on days 0.5–14, but the magnitude of this effect was diminished after 10 days (Table 2). The bacterial assemblage was dominated for most of the study by smaller cells (<0.4 μm), but this proportion changed over time and was

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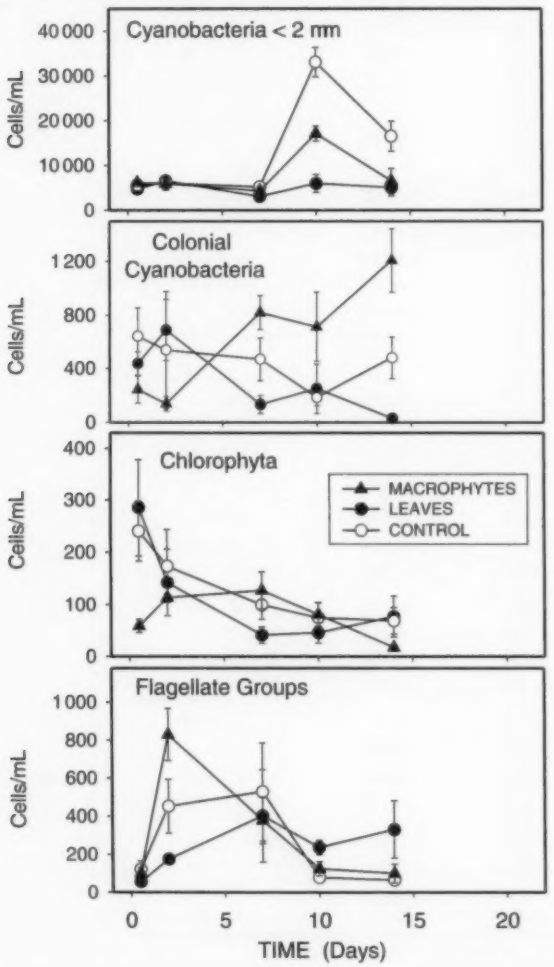

Fig. 5. Densities of phytoplankton cells classified into four major algal groups (based on abundance) in Calder Lake mesocosms amended with different C sources (flagellate category includes Chrysophyta, Pyrrophyta, and Cryptophyta) (macrophyte detritus added, terrestrial detritus (leaves) added); means ± 1 SE.

affected by treatment. Initially, about 85% of bacterial cells in all systems were <0.4 μm in length, but this proportion gradually declined to about 50% in controls by day 21. In mesocosms supplied with macrophyte detritus the proportion of small cells first increased to 92% by day 2 and then declined to 65% by day 7 and further to about 40% after day 10. Systems supplied with terrestrial detritus showed no increase in small cells early in the study, but exhibited a similar final percentage of smaller cells (43%) by day 21.

Heterotrophic nanoflagellates exhibited an increase in all communities early in the study (day 2), with significantly greater numbers in systems receiving macrophyte detritus (2.2 times greater than controls; HSD, $P < 0.05$; Table 2) and then steep declines in all systems (no samples collected on day 21). The four most abundant representatives of heterotrophic nanoflagellate assemblages were *Goniomonas*, *Heteronema*, *Bodo*, and unidentified choanoflagellate species. Autotrophic nanoflagellates followed nearly the opposite trajectory from heterotrophic nanoflagellates and responded differently to detritus treatments. Autotrophic nanoflagellates, mainly *Chrysochromulina parva* and *Ochromonas* sp., exhibited a steep increase in control mesocosms only (effects significant on days 7 and 14; Table 2). The overall response of phytoplankton biomass (as Chl *a*) to organic C treatments was complex (Fig. 4). Initially (day 2) and later in the experiment (days > 10), mesocosms receiving macrophyte C exhibited significantly greater Chl *a* concentrations than either controls or terrestrial C amended systems (Table 2). By day 7, control communities contained 65% and 2.7-fold more Chl *a* than macrophyte and terrestrial leaf amended systems, respectively. A brief Chl *a* peak in systems receiving terrestrial C (day 10) was not significantly different from other systems (Table 2).

The complexity of the general Chl *a* response is partially explained by enumeration of algal cells. Groups and species of algae responded differently to the treatments applied (days 0.5–14 only; Fig. 5). Abundances of picoplanktonic cyanobacteria (<2 μm) increased by midexperiment (day 10), with numbers greatest in controls (3.3×10^5 cells/mL) and least in mesocosms amended with terrestrial C (0.6×10^5 cells/mL). Macrophyte detritus had an apparent inhibitory effect on these organisms (about 52% of control). Densities in all three treatments were significantly different from each other (Table 2). Unlike smaller cells, numbers of colonial cyanobacteria (especially *Coelosphaerium naeglianum*, *Anabaena* sp., *Dactylococcopsis acicularis*) declined over time in control and terrestrial C amended systems, but increased steadily in mesocosms with macrophyte C (significantly greater on day 14; HSD, $P < 0.05$). No obvious or statistically significant effects were observed with green algal species; all common species (especially *Sphaerocystis schroeteri*, *Oocystis* sp.) declined over time regardless of treatment. An early peak (day 2) in the numbers of several algal flagellates (Chrysophyta, Cryptophyta, Pyrrophyta) was observed, although treatment differences were attributable to an apparent inhibition in mesocosms supplied with terrestrial C (significantly less than in controls and macrophyte C amended systems) rather than stimulation by macrophyte detritus, unlike what was observed for other planktonic organisms. Changes in total numbers was mainly the result of lesser abundances of the dinoflagellate *Peridinium gatunense* and an unidentified nonscaled chrysophyte (8–9 μm) in systems receiving terrestrial C. Later, numbers declined in all treatments, but a significantly greater number of algal flagellates were observed on day 10 in systems amended with terrestrial C, threefold greater than controls and 2.2-fold greater than in mesocosms with macrophyte C (HSD, $P < 0.05$; Table 2). This was primarily the result of greater numbers of *Synura* sp., *Cryptomonas* sp., and *Ceratium hirundinella* during later sampling periods in terrestrial POC systems.

Effects on zooplankton

The rotifers *Keratella cochlearis* and *Polyarthra vulgaris* and copepod nauplii were the most abundant members of the zooplankton community during the study (days 0.5–14 only). All three taxa were apparently stimulated by macrophyte C, but the timing of its effect varied by species

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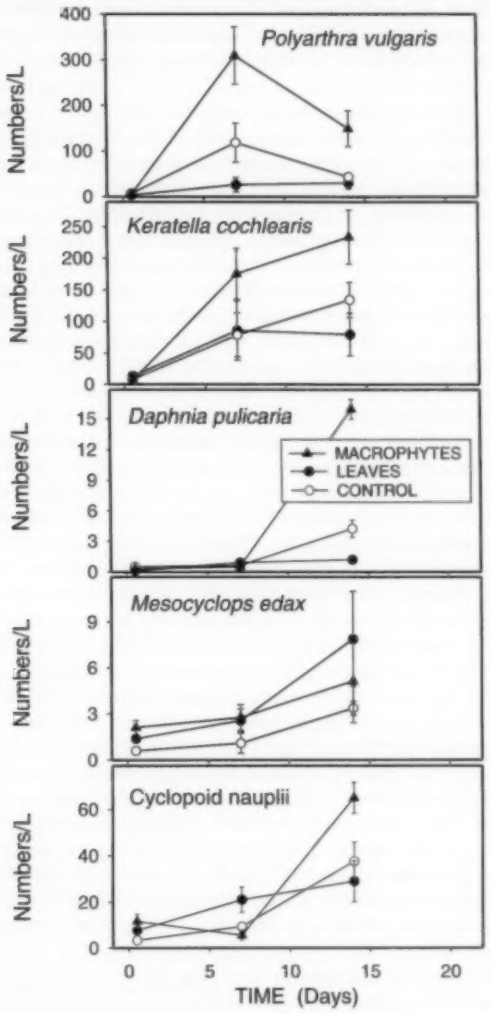
Fig. 6. Densities of common zooplankton species in Calder Lake mesocosms amended with different C sources (macrophyte detritus added, terrestrial detritus (leaves) added); means ± 1 SE.



(Fig. 6). Population size in *Keratella cochlearis* increased over time in all treatments, but was 1.7-fold greater in systems receiving macrophyte C than controls by day 14 (HSD, $P < 0.05$; Table 3). At the same time, *Keratella* numbers in mesocosms amended with terrestrial C were reduced to <60% those of controls. Another common rotifer, *Polyarthra vulgaris*, reached maximum numbers in all systems by day 7, with a similar pattern of significantly greater abundances with macrophyte C (2.6 times controls; HSD, $P < 0.05$) and significantly reduced numbers in terrestrial C mesocosms (22% of controls; $P < 0.05$). By day 14, densities had decreased in all systems, but macrophyte C amended systems still supported significantly greater numbers of *Polyarthra* (4.3 times controls). Abundances of less common rotifers (*Conochilus* sp., *Trichocerca* sp., *Brachionus* sp.) were not significantly affected by organic C treatments (data not shown). Copepod nauplii also reached

Table 3. Effects of POC treatments on abundances of selected zooplankton taxa in Calder Lake mesocosms measured on days 2–14.

Day	Variable	*F* score	*P*	Individual effects
2	*Keratella*	nd		
7		2.20	0.127	L ≈ M ≈ C
10		nd		
14		4.93	0.036	M > L ≈ C
2	*Polyarthra*	nd		
7		13.29	0.002	M > C > L
10		nd		
14		11.23	0.004	M > C ≈ L
2	*Daphnia*	nd		
7		1.44	0.252	L ≈ M ≈ C
10		nd		
14		16.273	0.001	L > M ≈ C
2	*Mesocyclops*	nd		
7		1.33	0.279	L ≈ M ≈ C
10		nd		
14		0.92	0.432	L ≈ M ≈ C
2	Copepod nauplii	nd		
7		1.82	0.217	L ≈ M ≈ C
10		nd		
14		5.65	0.026	M > L ≈ C

Note: $n = 4$ for each day; no samples on day 21. See Table 1 for further details.

maximum numbers later in the study. Macrophyte C treatments resulted in 1.7 times greater numbers than in controls (HSD, $P < 0.05$). The most abundant larger zooplankters were *Daphnia pulicaria* and *Mesocyclops edax* (Fig. 6). Other less common representatives included *Chydorus* sp., *Diacyclops* cf. *thomasii*, and *Skistodiaptomus* cf. *pallidus*; their abundances were not obviously affected by POC treatments. Abundances of *Daphnia pulicaria* remained low in all systems until day 14 (<1 organism/L), when numbers increased in macrophyte POC and control mesocosms by 32- and 7-fold, respectively; abundances in macrophyte C amended systems were 3.8 times greater than in controls (HSD, $P < 0.05$), while numbers were only 28% of controls in terrestrial C amended systems (HSD, $P < 0.05$). Numbers of the copepod *Mesocyclops edax* also increased over time, but unlike other zooplankton taxa, densities were more than twofold greater in systems amended with terrestrial C than controls; differences, however, proved to be nonsignificant.

Discussion

This study demonstrates that small inputs of detrital C can have significant and complex effects on a plankton community. A key result was that the source of DOM can influence phytoplankton production and species composition, as well as microbial activity. More broadly, these data suggest that C source and quality may be important determinants of both microbial and "classical" plankton community structure.

Phytoplankton numbers and biomass (as Chl *a*) exhibited positive and negative responses to treatments, patterns that often differed from those observed for bacterioplankton. This variability undoubtedly reflects the nutritional diversity within the phytoplankton community. Many species of

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cyanobacteria declined in response to the addition of terrestrial C, while numbers of some algal flagellates (potential mixotrophs) such as *Ceratium hirundinella*, *Cryptomonas* sp., and *Synura* sp. increased. Similarly, addition of humic material to limnetic enclosures in a meso-oligotrophic lake in southern Finland stimulated the growth of a *Cryptomonas* species during summer (Arvola et al. 1996). However, abundances of other possible mixotrophs in Calder Lake, such as *Chrysochromulina parva* and *Peridinium gatunense*, declined following addition of terrestrial detritus. What chemical or biological factors may be causing these declines is not clear. Size fractionation studies of DOM in Lake Mekkojarvi (Finland) indicate that lower molecular weight (<1000) DOM may be inhibitory to the growth of several algal flagellate species (Tulonen et al. 1992). Future experiments need to test the idea that both the quantity and quality of DOM in aquatic systems may alter specific nutritional activities (growth, photosynthesis, phagotrophy) of algal flagellates.

An equally complex response by bacteria in Calder Lake mesocosms points to a second key finding. Unlike simple inorganic nutrient pulses, the addition of detrital C initiated a sequence of chemical and biological events that continued to change as organic matter was decomposed. For example, the sustained depression of O_2 levels in both sets of detritus-amended systems (Fig. 1) suggests that POC was degraded gradually by microorganisms, while supplying inorganic nutrients (e.g., significant P increases) to primary producers. Because organic matter was supplied in particulate form, transformations of POC to DOC likely required longer periods before bacterial assimilation was possible. Despite significant O_2 declines in both POC treatments, bacterial numbers were stimulated only by submersed macrophyte detritus and were inhibited by a similar dose of terrestrial C (Fig. 4; Table 2). These results suggest that bacterial decomposition of macrophyte and terrestrial POC and DOC was proceeding by different routes.

It has been assumed that a fraction of the allochthonous DOC pool in lakes is not recalcitrant (Tranvik 1992). In order for this labile fraction to stimulate bacterial growth in Calder Lake, the sum of any possible negative effects (e.g., greater bacterivory, reduced light penetration, inhibitory compounds) must be less than the stimulatory effects (e.g., greater DOC, P, N) provided by the input of DOM. Food web factors (e.g., grazing) are unlikely to have caused these declines in bacterial densities because the numbers of heterotrophic nanoflagellates and rotifers in mesocosms amended with terrestrial C were not significantly different from controls (Figs. 4 and 5; Tables 2 and 3).

Optical properties were also affected significantly by small additions of DOM. We recorded significant increases in UV and OD_{440} absorbance in Calder Lake water, although maximum levels were <25% those measured in moderately colored systems (De Hahn and De Boer 1987; Lindell et al. 1995). The goal of the present study was not to create a humic-stained lake, but to examine potential effects of realistic detritus additions to small, uncolored lakes. Evidence that treatments were subtle is also found in relatively low OD_{440} (≈ humic fractions: Kirk 1983) values that were <20% that measured in mesohumic Lake Sjätesjön, Sweden (Lindell et al. 1995). Ratios of OD_{250} to OD_{365}, an index of low molecular weight compounds, were two- to three-fold greater in Calder Lake mesocosms than in lakes with a substantial input of allochthonous organic matter (De Hann and De Boer 1987). If DOM treatments indeed supplied proportionately greater amounts of low molecular weight compounds to Calder Lake water, our data support prior studies suggesting inhibitory effects on phytoplankton growth (Tulonen et al. 1992).

A third important result was that macrophyte detritus stimulated many more components of the plankton community in Calder Lake than did terrestrial detritus. Only macrophyte C addition resulted in significantly greater numbers of bacterioplankton, phytoplankton, heterotrophic nanoflagellates, and several zooplankton species. These effects are not likely due to the release of P during decomposition because terrestrial DOM also increased P supply by a similar amount. Thus, while bacteria can be stimulated by inorganic P subsidy (e.g., Toolan et al. 1991), the positive effects seen in this study (with only macrophyte DOM) suggest that C lability may be the primary mechanism. We have some limited data to support this claim. Average C to N ratios of particulate matter (detritus plus plankton) collected from mesocosms supplemented with terrestrial DOM (9.88 ± 0.15) were nearly twice those in mesocosms with macrophyte DOM (5.48 ± 0.34) (J.D. Wehr and M.M. MacDonald, unpublished data). A higher C:N value is one index of more recalcitrant organic matter (Kristiensen 1994). In one freshwater littoral community, bacterial production varied inversely with the C:N of plant detritus when used as a primary substrate (Moran and Hodson 1989).

While C source had profound effects on bacterioplankton growth, our data also provide partial evidence of cascading trophic effects through the community. The fact that macrophyte DOM (through C and P) stimulated bacterioplankton and higher trophic levels suggests that the Calder Lake microbial loop may be at least partly bottom-up controlled. Our earlier studies with inorganic nutrients have shown similar results (Toolan et al. 1991; Le et al. 1994). There is some evidence of top-down control as well. Early in the experiment (day 2), heterotrophic nanoflagellate abundance peaked, which was followed by increases in rotifers (days 7–10) and, later, larger zooplankton (day 14). Bacterial abundances began to decline by midexperiment (day 7: 10%; day 10: 60%), suggesting that increases in micrograzers may have regulated their numbers. Larger bacterivores (e.g., rotifers) may selectively graze larger (i.e., growing, dividing) cells, while protists may select smaller cells, hence influencing numbers and size structure (Holen and Boraas 1991). In Calder Lake assemblages, smaller bacterial cells (<0.4 μm) dominated initially, but their proportion increased by midexperiment in mesocosms supplied with macrophyte detritus, until a rapid decline followed increases in heterotrophic nanoflagellate and rotifer densities. In contrast, in mesocosms with terrestrial C, we observed lower and fairly stable protist and rotifer densities.

The responses of larger zooplankton to treatments were less clear. By day 14, densities of *Daphnia pulicaria* and copepod nauplii in macrophyte C amended systems increased to more than twice the densities measured in controls (Fig. 6). If phytoplankton forms the primary food source for cladocerans and copepods in this lake, then one would predict that an earlier pulse in Chl *a* should have trig-

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gered this response. Indeed, the greatest Chl *a* concentration was measured in communities supplemented with macrophyte C just after the rise in macrozooplankton densities. It has been proposed that linkages between the microbial and classical food webs may occur at the zooplankton–protist interface (Sherr and Sherr 1994). It is possible that cladocerans may also have been feeding on (bacterial-feeding) protists, many of which are in a similar size range of many phytoplankton species. Cladocerans may have direct, but dual impacts on bacterioplankton, stimulating numbers by releasing organic matter during phytoplankton grazing and negatively through grazing.

The present study aimed to characterize the effects of organic C source, separate from concentration, on a planktonic community. Our first hypothesis, that macrophyte and terrestrial detritus would enhance bacterioplankton production, was based on the idea that any increase in DOC would stimulate bacterial growth, although any effects of more recalcitrant C would be seen later in the study. This hypothesis was only partially supported by the data, perhaps because decomposition of terrestrial C to simpler, more labile forms may have required longer biological processing time and perhaps greater exposure to UV irradiance (Wetzel et al. 1995). As OD_{250} and OD_{440} in terrestrial C amended systems remained relatively stable and greater than controls, while bacterial numbers declined, we have no evidence of any substantial natural photolysis of DOM.

The source of DOM elicited different responses at several levels within the community. Autotrophic protists peaked only in controls, suggesting that they may have been functioning as strict autotrophs in those systems, while planktonic bacteria and heterotrophic flagellates increased in response to the addition of macrophyte DOM. Some mixotrophic flagellates increased, while others declined, in systems amended with terrestrial detritus. Complex responses such as these to substrate manipulations require that microbial food web models include multiple routes for materials, especially as many microorganisms and other organisms may have several functional roles within freshwater plankton communities.

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PERSPECTIVES

A general, life history based model for regional management of fish stocks: the inland lake trout (*Salvelinus namaycush*) fisheries of Ontario[1]

B.J. Shuter, M.L. Jones, R.M. Korver, and N.P. Lester

Abstract: Life history characteristics of 54 Ontario lake trout (*Salvelinus namaycush*) populations vary with differences in lake area (range 25–450 000 ha) and total dissolved solids (TDS) (range 15–180 mg·L^{-1}). Populations from large lakes exhibit greater maximum sizes, greater ages and sizes at first maturity, lower natural mortality rates, and lower sustainable yields. Populations from high-TDS lakes exhibit higher growth rates in early life, lower ages at first maturity, larger sizes at first maturity, and higher natural mortality rates. Angler catchability increases significantly at low population densities. With these relationships included in an age-structured population model, we found that the fishing mortality rate at maximum equilibrium yield ranges from 0.12·year^{-1} for a 100-ha, low-TDS lake to 0.37·year^{-1} for a 10 000-ha, high-TDS lake; the fishing effort level at maximum equilibrium yield ranges from 6.6 angler-h·ha^{-1}·year^{-1} for a 100-ha, low-TDS lake to 4.0 angler-h·ha^{-1}·year^{-1} for a 10 000-ha, high-TDS lake. Populations from small, low-TDS lakes are more sensitive to overexploitation than populations from large, high-TDS lakes. Easily measured, environmental correlates of life history characters may be common among fish species and are useful in developing exploitation guidelines for populations that are not well studied.

Résumé : Certaines caractéristiques du cycle vital de 54 populations de touladi (*Salvelinus namaycush*) de l'Ontario varient en fonction de la superficie des lacs (fourchette de 25 à 450 000 ha) et des solides dissous totaux (SDT) (fourchette de 15 à 180 mg·L^{-1}). Les poissons des populations des grands lacs ont des tailles maximales plus élevées, sont plus âgés et plus gros à la première maturité, ont des taux de mortalité naturelle moins élevés et donnent des rendements équilibrés inférieurs. Les poissons des populations des lacs à forte teneur en SDT ont dans leurs premiers stades des taux de croissance plus élevés, sont plus jeunes et plus gros à la première maturité et ont des taux de mortalité naturelle plus élevés. Le potentiel de capture par les pêcheurs sportifs s'accroît de façon significative quand les densités des populations sont faibles. En incluant ces relations dans un modèle de population structuré selon l'âge, nous avons établi que le taux de mortalité par pêche au rendement maximal équilibré varie de 0,12·an^{-1} pour un lac de 100 ha à faible teneur en SDT à 0,37·an^{-1} pour un lac de 10 000 ha à forte teneur en SDT; l'effort de pêche au rendement maximal équilibré varie de 6,6 h-pêcheurs·ha^{-1}·an^{-1} pour un lac de 100 ha à faible teneur en SDT à 4,0 h-pêcheurs·ha^{-1}·an^{-1} pour un lac de 10 000 ha à forte teneur en SDT. Les populations des petits lacs à faible teneur en SDT sont plus sensibles à la surexploitation que les populations des grands lacs à forte teneur en SDT. Des corrélats environnementaux facilement mesurables de paramètres du cycle vital peuvent être communs à des espèces de poisson et sont utiles pour l'élaboration de lignes directrices en matière d'exploitation des populations qui ne sont pas bien étudiées.

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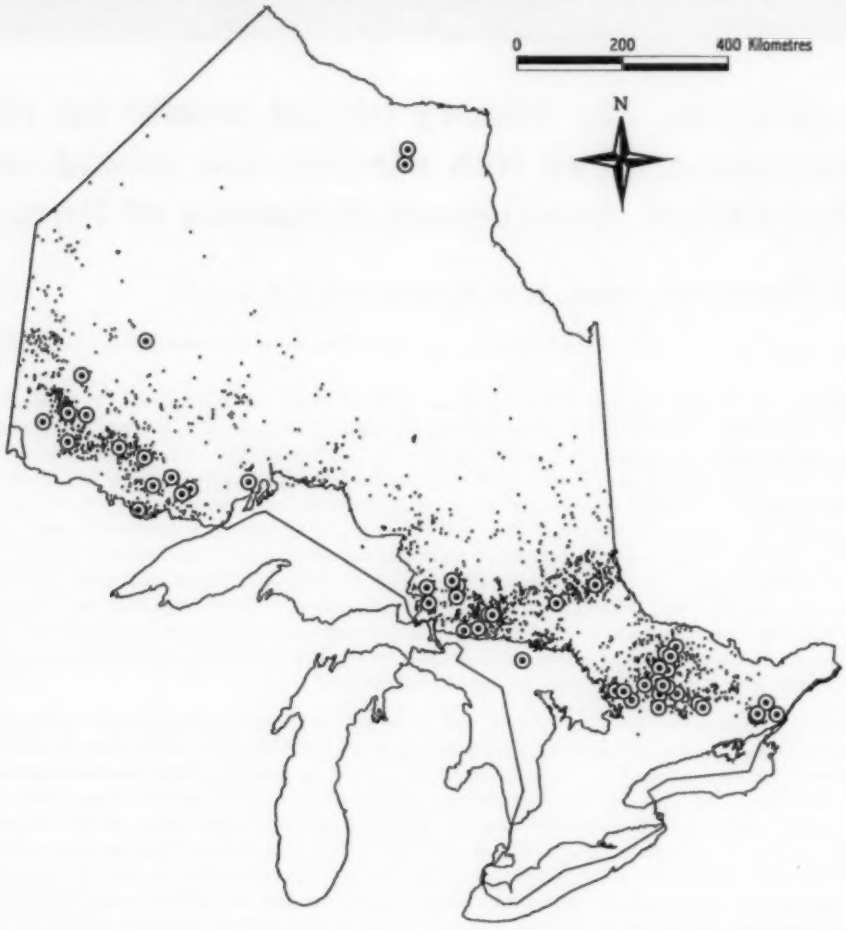
Fig. 1. Locations of about 2200 lake trout lakes in Ontario; the 54 lakes sampled for this studied are circled.



Introduction

The principal challenge of managing exploitation of fish in the numerous inland lakes of eastern North America lies in the problem of generalization. Overall, the size and economic importance of the resource is large, but it is divided into so many small, isolated populations (Fig. 1) that it is impractical to carry out detailed stock assessments on each of them, and this, in turn, makes it impossible to manage these populations on a lake by lake basis. As a result, we are faced with the necessity of generalizing about sustainable levels of exploitation for all populations on the basis of knowledge from a very limited sample of populations. In this paper, we develop a general approach to this problem by examining the degree of demographic variation in a relatively large and representative sample of Ontario lake trout (*Salvelinus namaycush*) populations and exploring the implications of this variation for developing effective strategies for regional management of exploitation.

Recognition of the challenge of generalization is not new. Development of empirical models to predict potential fish yield from individual lakes began in the 1940s (reviewed by Leach et al. 1987). The most well-known and widely utilized model of this type is the morphoedaphic index (MEI: Ryder et al. 1974). The success of such models hinges on the fact that measures of nutrient availability, or trophic state, are repeatably correlated with indices of lake-wide fish production (at least to within an order of magnitude). More recent investigations have shown that species-specific yields can be predicted from climate variables (Schlesinger and Regier 1983) and from measures of the amount of thermally suitable habitat (Christie and Regier 1988). These relationships can be used to predict the yield potential of a lake from a relatively easily measured set of lake characteristics. However, they do not allow prediction of sustainable levels of fishing mortality or effort. This requires a model based on explicit descriptions of both the demographics of the exploited populations and the relationship between fishing mortality and fishing effort. Our objective is to develop such a model for managing angling exploitation of lake trout on the inland lakes of Ontario.

The lake trout is the only major indigenous sport fish species adapted to the deep, cold waters of oligotrophic lakes. It exists in about 2200 inland Ontario lakes (Fig. 1) and is fished during winter, spring, and summer seasons. Lake trout are characterized by slow growth, late maturity, low repro-

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ductive potential, and a slow replacement rate. These attributes make the species especially vulnerable to overexploitation. For lake trout populations in the southern part of the province, levels of fishing effort are often high, harvests often exceed annual production, and the quality of many fisheries is in decline (Evans et al. 1991). Populations in northern Ontario are in better condition, but similar trends are expected in the future if angling effort (E, angler-hours per hectare) and fishing mortality rate (F, per year) are not held within appropriate bounds.

Recent work on both freshwater (brook trout *Salvelinus fontinalis*: Hutchings 1993, 1996) and marine fish species (Atlantic cod *Gadus morhua*: Myers et al. 1997) has demonstrated that interpopulation variation in demographic parameters can be large enough to affect intrinsic rates of population growth and hence influence the sensitivity of individual populations to exploitation. In several studies, significant portions of this variation were associated with simple habitat characteristics (e.g., water temperature: Myers et al. 1997; conductivity/fish community: Trippel and Beamish 1989, 1993). We begin our study by demonstrating that the life history parameters for a lake trout population can be predicted from simple environmental characteristics of the lake that it lives in. We use these relationships to construct a population model that can be calibrated to individual lakes with minimal lake-specific information. We go on to define empirically the relationship between fishing mortality and angling effort for Ontario lake trout angling fisheries and embed that relationship in our model, thus creating a structure that explicitly acknowledges the respective roles that life history, habitat, and the fishery play in determining abundance, harvest, and sustainable levels of exploitation. We use this model to predict sustainable levels of fishing mortality and fishing effort for typical Ontario lake trout lakes. Finally, we identify and discuss those aspects of our approach that may be applicable to other species in different geographic regions.

Methods

Population yield model

To identify the parameters needed to predict yield as a function of fishing mortality, we developed an age-structured equilibrium yield model. It was derived by combining a generalized stock–recruitment relationship (Shepherd 1982) with conventional yield-per-recruit and biomass-per-recruit functions (Appendix 1). The result is a yield equation of the following form:

$$Y = f(W_\infty, \omega, t_m, f_{max}, \alpha_{max}, M; \beta, B_0; t_c, F)$$

where the parameters can be grouped as follows.

(*i*) Life history parameters, defining rates of growth, mortality, and reproduction, characteristic of specific habitats in the absence of intraspecific competition: W_∞, asymptotic mass of an adult fish (kilograms); ω, rate of length growth (centimetres per year) in early life (Gallucci and Quinn 1979), the product of the von Bertalanffy growth parameters K (per year) and L_∞ (centimetres); t_m, age of maturation, knife-edge transition to maturity assumed; f_{max} fecundity, number of eggs per kilogram for a mature female at low population density; α_{max}, survival from egg to age 1 at low population density; M, instantaneous natural mortality rate (per year) for fish aged 1 year and over.

(*ii*) Habitat quantity parameters, defining the rates at which early survival and (or) fecundity are reduced from their maximal values as the population fills available habitat and approaches its carrying capacity: β, a parameter that sets the rate at which early survival and (or) fecundity declines as the population approaches its carrying capacity (i.e., a higher β value produces a more rapid decline); B_0, a scaling parameter (kilograms per hectare) that reflects the amount of habitat available to the population, and hence is directly related to both the carrying capacity of the population and its maximum sustainable harvest.

(*iii*) Fishery parameters, defining the part of the population that is exploited and the intensity of exploitation: t_c, age of first capture by the fishery, knife-edge transition to full vulnerability assumed; F, instantaneous fishing mortality (per year) applied to all fish aged $\geq t_c$.

Each of these parameters must be estimated to characterize the model for a specific lake. Our objective is to find a basis for estimating their values for lakes lacking extensive stock assessment information. To address this objective, we examined existing data on Ontario lake trout populations with two questions in mind. (1) Are there empirical relationships that link any of the yield model parameters to some readily measured lake attribute such that those parameters could be predicted from that attribute and would not have to be measured directly? (2) Do the life history parameters covary in a consistent fashion among lake trout populations such that an estimate of the value for at least one parameter could be used to predict the values of others?

Our data come from a set of 54 lakes (Table 1) widely distributed throughout Ontario (Fig. 1) and include estimates of growth rates, mortality rates, maturation schedules, and fecundity. Much of this information was assembled as part of a concerted effort by the Ontario Ministry of Natural Resources to summarize existing knowledge pertinent to the management of Ontario's inland lake trout populations (Payne et al. 1990).

Describing lifetime growth pattern

We chose to describe the lifetime growth pattern of lake trout in terms of the von Bertalanffy equation, parameterized according to the scheme first suggested by Gallucci and Quinn (1979):

$$L_t = L_\infty \cdot (1 - e^{-(\omega/L_\infty)\cdot t})$$

We chose the von Bertalanffy equation because it is simple, it typically provides superior fits to fish growth data (Chen et al. 1992), and it has a form that is very similar to the form predicted by detailed growth models that explicitly describe the accumulation of energy and its optimal allocation to activity, somatic growth, and reproductive products (Roff 1983; Kozlowski 1996). We chose the Gallucci–Quinn parameterization because the two parameters (ω, L_∞) have tangible units (e.g., centimetres per year, centimetres) and are easily interpretable, statistical estimates for both parameters are not intrinsically intercorrelated, as are the traditional von Bertalanffy parameters K and L_∞ (Chen et al. 1992), and the parameters cleanly separate lifetime growth data into the growth rate prior to sexual maturity (ω) and an index of the amount of growth that occurs after maturity (L_∞); this separation satisfies the objections raised by authors who have criticized use of the von Bertalanffy equation in life history studies (Roff 1984; Day and Taylor 1997).

Results

Parameter estimates for Ontario lake trout populations

Growth (ω, W_∞)

Payne et al. (1990) observed that the lifetime growth patterns of lake trout populations are correlated with lake area. To further explore this relationship, we summarized each of the growth patterns in our 54 lakes with two parameter esti-

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Table 1. Summary of the lakes used to explore relationships among lake trout demographic and fishery parameters.

Lake	Surface area (ha)	TDS ($mg \cdot L^{-1}$)	Aging method	No. of fish aged	L_∞ (cm)	ω ($cm \cdot year^{-1}$)	Z ($year^{-1}$)	Maturity	Vulnerability	Catchability
Adelaide	137	28	S	299	43.8	12.75	—	—	—	—
Basswood (Wakwekobi)	2 709	—	S	278	74.8	13.02	1.57	—	×	×
Big Clear	187	50	S	264	47.9	11.54	0.52	×	×	×
Big Rideau	5 761	138	S	270	62.1	14.47	0.49	×	×	×
Big Salmon	148	58	S	225	59.4	9.80	0.39	—	×	×
Bone	121	30	S	708	43.0	10.75	1.08	×	×	×
Boshkung	716	35	S	132	63.2	7.84	—	—	—	—
Buddell	194	28	O	64	62.2	6.53	0.12	—	—	—
Burnt Island	110	25	O	87	61.0	7.93	—	×	—	—
Charleston	2 517	137	S	285	64.9	11.75	0.64	×	×	×
Chiblow/ Denmans	2 725	31	S	1255	70.6	11.79	1.11	×	×	×
Christman (Jim Christ)	59	46	S	204	50.9	15.42	1.03	×	×	×
Devil	1 062	109	S	272	58.8	11.64	0.44	—	×	×
Dickey	214	90	S	240	40.4	14.34	0.93	×	×	×
Eagle	27 691	39	S	35	78.4	9.02	—	—	—	—
Flack	951	22	S	2142	64.0	10.62	1.06	×	×	×
Gong	385	24	S	79	81.2	10.31	—	—	—	—
Goulais	240	31	S	111	54.0	9.83	—	—	—	—
Greenwater	3 060	44	O	189	74.0	8.07	—	—	—	—
Greenwich	484	20	O	283	50.8	7.16	—	—	—	—
Gull	995	30	S	447	54.5	8.94	—	—	—	—
Happy Isle	536	23	S	2993	58.2	9.14	—	—	—	—
Hawley	1 235	134	O, F	451	63.1	8.77	0.18	—	×	—
Islets	189	16	O	100	57.8	9.08	—	×	—	—
Kawagama	2 819	22	S	155	48.8	12.01	—	—	—	—
Kennisis	1 417	23	S	312	56.2	9.16	—	—	—	—
Lake 223	30	—	F	799	46.0	16.10	0.38	—	—	—
Lake 224	25	—	F	522	41.0	12.30	0.36	—	—	—
Lake Joseph	5 156	45	S	602	72.0	8.06	—	—	—	—
Lake Lavieille	2 402	32	S	569	79.2	9.19	—	×	×	×
Lake Louisa	490	23	S	571	49.5	11.73	0.90	×	×	×
Lake Manitou	10 461	180	K	—	73.0	20.44	0.22	×	—	—
Lake Muskoka	12 215	61	S	133	66.0	12.41	—	—	—	—
Lake Nipigon	448 060	102	O	364	90.3	16.53	—	×	×	—
Lake Opeongo	5 860	29	S	6015	87.2	8.02	0.43	×	×	×
Lake Rosseau	6 374	33	S	111	64.9	12.98	—	—	—	—
Lake Temagami	20 972	49	S	344	79.3	10.86	0.67	—	×	×
Lake of Bays	6 904	23	S	51	68.8	8.46	—	—	—	—
Lake of the Woods (shoal)	25 856	138	O	199	81.5	11.17	—	×	—	—
Little Gull	325	16	O	67	52.5	10.82	—	—	—	—
Little Joe	221	52	O	97	61.9	12.69	—	×	—	—
Loch Erne	165	67	O	118	68.4	10.60	—	×	—	—
Miskwabi	264	62	S	94	55.8	10.77	—	—	—	—
Nelson	316	32	S	410	61.9	9.35	—	—	—	—
Pickerel	6 059	20	O	174	59.6	8.52	—	—	—	—
Ranger	2 254	25	S	251	55.5	13.04	0.69	—	×	×
Redstone	1 130	64	S	80	48.8	9.91	—	—	—	—
Robinson	421	15	O	48	59.8	7.65	—	×	—	—
Semiwhite	304	28	S	605	61.1	10.26	1.07	—	×	×

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Table 1 (*concluded*).

Lake	Surface area (ha)	TDS ($mg \cdot L^{-1}$)	Aging method	No. of fish aged	L_∞ (cm)	ω ($cm \cdot year^{-1}$)	Z ($year^{-1}$)	Maturity	Vulnerability	Catchability
Smoke	607	25	S	282	76.3	8.39	—	×	—	—
South Otterskin	214	115	O	91	63.9	14.63	—	×	—	—
Squeers	384	28	O	1009	55.0	7.54	0.39	×	—	×
Sutton	3 764	154	O, F	188	63.7	9.24	0.15	—	×	—
Wollaston	363	162	S	153	40.2	10.37	—	—	×	—

Note: The ×'s in the last three columns indicate which lakes were used to examine a particular parameter. All 54 lakes were used in the lake size and growth analyses. Aging methods: S, scale; O, otolith; F, fin ray; K, known (clips). Estimates of total instantaneous mortality rate (Z) were obtained from catch at age data (Chapman and Robson 1960; Robson and Chapman 1961).

mates, ω and L_∞, with ω estimated as the product of the standard von Bertalanffy growth parameters K and L_∞ (Galucci and Quinn 1979). Estimates for 10 of these lakes were taken from Trippel and Beamish (1989). Estimates for the remaining 44 lakes were derived using the methods outlined below.

For most populations, age determination was done using scales, otoliths, or fin rays. For populations with scale ages only, mean size at age 8 and older was probably biased high (Casselman 1983). This bias would lead to a strong positive bias in L_∞ estimates derived from methods that involve extrapolating size at age curves to infinite age (e.g., direct nonlinear fit of the von Bertalanffy equation) or extrapolating annual size increments to the point where they vanish (e.g., Walford linear regression). To avoid these biases, we followed Pauly's (1984) approach and estimated L_∞ as the mean length of the 10 largest fish sampled from the lake. For 13 of our 54 lakes, we had accurate size at age data from otoliths over a range of ages large enough to permit us to estimate L_∞ by a nonlinear fit of the von Bertalanffy equation to the data. Estimates of L_∞ derived using this method were highly correlated ($r^2 = 0.81$, $p \leq 0.0001$) and similar to estimates derived using the Pauly approach. We also checked for negative bias in the Pauly estimates of L_∞ for populations with high total mortality (Z) rates, where larger older fish might be rare. For the 23 populations with both L_∞ and Z estimates (range of Z estimates 0.15–1.6; see Table 1), there was no significant correlation ($r^2 < 0.001$, $p > 0.90$) between the two variables and thus no indication of bias.

All size at age data were derived from harvest samples. To avoid potential biases inherent in such samples, K was estimated using size at age data for ages that were old enough to be well represented in the fishery, but young enough to be reliably aged. This was done by fitting the following equation to observed, lake-specific data on length at age (L_i) using simple linear regression:

$$-\ln\left(1-\frac{L_i}{L_\infty}\right)=K\cdot i$$

derived by rearranging the conventional von Bertalanffy equation and setting $t_0 = 0$. If ages were obtained from scales, we based our K estimate on size at age for ages 4–7. If ages were obtained from otoliths or fin rays, we used size at age data for all ages >4 because lake trout otolith and fin ray ages are reliable over a very broad age range, while scale ages are reliable to age 8 (Casselman 1983).

With lifetime growth pattern summarized in terms of ultimate size (L_∞) and early growth rate ($\omega = K \cdot L_\infty$), we found that a pair of empirical relationships could be used to predict these parameters from two simple lake characteristics.

(*i*) A log–log regression analysis ($r^2 = 0.459$, $p < 0.001$, $n = 54$) of the association between L_∞ and lake area (median = 661 ha, range 25.4 – 44 810 ha) generated the following equation for predicting L_∞:

$$(1) \qquad L_\infty = 37.15 \cdot \text{area}^{0.071}$$

(*ii*) A log–log regression analysis ($r^2 = 0.227$, $p < 0.001$, $n = 51$) of the association between ω and total dissolved solids (TDS) (median = 34.3 $mg \cdot L^{-1}$, range 15–180 $mg \cdot L^{-1}$) generated the following equation for predicting ω:

$$(2) \qquad \omega = 5.60 \cdot \text{TDS}^{0.162}.$$

Parameter L_∞ was essentially independent of TDS and ω was essentially independent of lake size. Therefore, with TDS constant, asymptotic length will increase with lake size, early growth rate will remain constant, and growth differences among lakes will emerge only as fish reach older ages. In contrast, with lake size constant, early growth rate will vary with TDS, asymptotic length will remain constant, and growth differences among lakes will disappear as fish reach older ages.

Length–mass regression parameter estimates were available for 23 Ontario lake trout populations (Payne et al. 1990). Among-lake variation in these parameter estimates was not correlated with variation in other lake trout biological parameters, with lake area, or with TDS. Therefore, we used the provincial average length–mass relationship (Payne et al. 1990) to estimate W_∞ (kilograms) from L_∞ (centimetres):

$$(3) \qquad W_\infty = 5.87 \times 10^{-6} \cdot L_\infty^{3.18}.$$

Thus, the first two life history parameters of our model, W_∞ and ω, can be predicted from lake area and TDS.

Maturation (t_m)

Life history theory predicts that age and size at maturity should vary with lifetime growth pattern. We looked for such relationships in our data set using female length at maturity data from 22 lakes (Payne et al. 1990; Trippel 1993): 13 estimates of length (or age) at which 5% of females are mature, 12 estimates of length (or age) at which 50% of females are mature, and 11 estimates of length (or age) at which 95% of females are mature. If only age at maturity

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data were available for a population, we used its von Bertalanffy growth equation to convert ages to lengths. We used analysis of covariance on these estimates of length at 5, 50, and 95% maturity (L_{m5}, L_{m50}, L_{m95}) to assess the relationship between size at maturity and lifetime growth pattern. With $\log(L_i, i = m5, m50, m95)$ as dependent variable, $\log(L_\infty)$ and $\log(\omega)$ as covariates, and 5% maturity, 50% maturity, and 95% maturity as treatments, we found that $\log(L_\infty)$, $\log(\omega)$, and the treatment variables each explained significant portions (overall multiple $R^2 = 0.725$, $p < 0.0001$, $n = 36$) of the observed variation in $\log(L_i)$. This analysis produced the following equation for predicting L_{m50}:

$$(4) \qquad L_{m50} = 1.56 \cdot \omega^{0.257} \cdot L_\infty^{0.665}.$$

Given L_{m50} for a particular lake, age at 50% maturity (t_m) can be estimated using the von Bertalanffy growth equation for the lake. Thus, we can estimate our third life history parameter, t_m, from lake area and TDS using the relationships that link these characters to L_∞, ω, and L_{m50}.

Early survival and fecundity (α_{max}, f_{max})

Few estimates are available for age 0 survival (α_{max}) of lake trout, but the few that exist are surprisingly consistent. In Lake Opeongo, Matuszek et al. (1990) found that age 0 survival at low population densities fell in the range from 0.0035 to 0.0055. M. Fruetel (Ontario Ministry of Natural Resources, Thunder Bay, Ont., personal communication) found a value of 0.00425 for Squeers Lake, a 400-ha lake in northwestern Ontario. For the period of lowest lake trout abundance in Lake Superior (prelamprey control), Ferreri et al. (1995) found a value of 0.00416, a value similar to that (0.004) by Walters et al. (1980) in an earlier assessment of Lake Superior lake trout. The mean of these four separate estimates is 0.0043.

Fecundity data were available for 13 lakes (Payne et al. 1990; Trippel 1993; E.A. Trippel, DFO Biological Station, personal communication). Although the range of values in this data set is quite wide (883–2226 eggs·kg body mass^{-1}), most estimates cluster around the mean value of 1506 eggs·kg body mass^{-1}.

In the results presented below, we have assumed fixed values of 0.0043 and 1506 for α_{max} and f_{max}, respectively. Given the sources of these values, we judge them to be representative of habitats where water quality parameters lie well within lake trout tolerance zones.

Natural mortality (M)

In his synthesis of demographic statistics from populations of 84 exploited fish species, Pauly (1980) found that M suffered by a population could be estimated from its von Bertalanffy growth parameters K and L_∞ plus an estimate of the annual average water temperature experienced by its members. We reanalysed his data set, generating a log–log regression equation that could use estimates for ω, L_∞, and water temperature to predict values for M ($R^2 = 0.718$, $n = 175$). Given an annual average water temperature of 6°C for lake trout (Shuter et al. 1987), we arrived at the following equation for predicting M:

$$(5) \qquad M = 2.064 \cdot \omega^{0.655} \cdot L_\infty^{-0.933}.$$

The water temperature estimate of 6°C was derived as follows: (*i*) in Lake Opeongo, the ice-free period is about 7 months long; for much of this time, the lake is stratified with an average hypolimnetic temperature of about 8°C; if we assume that lake trout occupy the hypolimnion during stratification (Martin and Olver 1980) and that water temperatures during ice cover are of the order of 3°C, then the annual average temperature that lake trout experience is about 6°C (Shuter et al. 1987) and (*ii*) values close to 6°C were calculated for our other lake trout lakes, using ice-free times and surface water warming rates estimated from the equations in Shuter et al. (1983).

Estimates of M, derived from eq. 5, were similar to independent, direct estimates of M (Table 2) obtained for eight Ontario lake trout populations as follows: (*i*) in six lakes, exploitation rates were effectively zero, and hence, we could assume that total mortality and natural mortality were equivalent; we estimated (Chapman and Robson 1960; Robson and Chapman 1961) total mortality from the average catch curve obtained from at least 3 years of catch at age data; data sets containing only 1 or 2 years of data were rejected because the presence of a single strong or week year-class in such a data record can produce severely biased mortality estimates (Ricker 1975) and (*ii*) for the more heavily exploited populations in Squeers and Opeongo, M estimates were obtained by subtracting estimates of fishing mortality from estimates of total mortality (Squeers: Ball 1988) or by estimating total mortality rates in years of very low fishing effort (Opeongo: Shuter et al. 1987).

Given this validation of M estimates based on eq. 5, we conclude that our sixth life history parameter (M) can be estimated from lake area and TDS using the relationships that link these characters to L_∞, ω, and M.

Habitat quantity (β and B_0)

A common assumption in both the fisheries literature (Ricker 1954; Cushing 1988) and the life history literature (Roff 1992; Charnov 1993) is that density-dependent effects on population growth rate act on the reproductive rate directly, either by increasing juvenile mortality or by reducing adult fecundity. This assumption is embodied in our population model through a stock–recruitment relationship that does not distinguish between effects on early mortality and fecundity. It simply assumes a negative effect of adult population density (B, kilograms of mature female biomass in the population per hectare) on the efficiency (maximum = $\alpha_{max} \cdot f_{max}$) with which mature female biomass produces 1-year-old fish:

$$N_1 = \frac{\alpha_{max} \cdot f_{max} \cdot B}{1 + \left(\frac{B}{B_0}\right)^{\beta}}.$$

Parameter B_0 establishes the range of population densities where significant declines in efficiency begin to be felt, and β defines the rate of decline as density increases through, and beyond, this range. We adopted the β value (2.0) used by Matuszek et al. (1990) in their long-term study of the Lake Opeongo lake trout population.

Parameter B_0 establishes the carrying capacity for a population and its value reflects the amount of limiting habitat

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Table 2. Estimates of lake trout natural mortality rates for eight Ontario lakes.

Lake	L_∞ (cm)	ω (cm·year^{-1})	Pauly M (year^{-1})	Observed M (year^{-1})	Data source	Comments
Opeongo	87.2	8.0	0.13	0.12	M from Shuter et al. 1987	M for 1964–1983 estimated by regression of Z on effort
Squeers	55.0	7.5	0.18	0.24	M from Ball 1988	M is average for 1982–1986 mark–recapture estimates
Hawley	63.1	8.8	0.18	0.18	K. Armstrong, Ontario Ministry of Natural Resources, Cochrane, Ont., unpublished data	M from catch curve for 1989–1991 data; exploitation light and assumed negligible
Sutton	63.7	9.2	0.18	0.15	K. Armstrong, Ontario Ministry of Natural Resources, Cochrane, Ont., unpublished data	M from catch curve for 1989–1991 data; exploitation light and assumed negligible
Manitou	73.0	20.2	0.27	0.22	Budd et al. 1968	M from 1959–1962 mark–recapture data; growth parameters fitted visually to line on graph in paper
Lake 223	46.0	16.1	0.36	0.40	K. Mills, Department of Fisheries and Oceans, Winnipeg, Man., unpublished data	M from catch curve for 1975–1978 data; unexploited lake
Lake 224	41.0	12.3	0.33	0.25	K. Mills, Department of Fisheries and Oceans, Winnipeg, Man., unpublished data	M from catch curve for 1976–1980 data; unexploited lake
Buddell	62.2	6.5	0.15	0.12	J. Casselman, Ontario Ministry of Natural Resources, Picton, Ont., unpublished data	M from catch curve; unexploited lake

Note: The Pauly M estimates were derived from $\log_e(M) = -0.0238 - 0.9326 \log_e(1.094\ L_\infty) + 0.6551 \log_e(\omega) + 0.4646 \log_e(T)$, where T is mean annual water temperature and L_∞ is measured as total length. This equation was derived by reanalysing the variation in M, in Pauly's (1980) collection of M, K, L_∞, and T values, in terms of ω, L_∞, and T. In deriving our Pauly M estimates for lake trout, T was fixed at 6°C and L_∞ values were multiplied by 1.094 to convert from fork length to total length. Sources for observed M estimates are described in the table. The estimate for Lake 223 was based on data collected in the time period before the population was impacted by experimental acidification.

available (Shuter 1990; Minns et al. 1996) for use by that population. The value of B_0 sets the absolute yields associated with specific fishing mortality rates (Appendix 1) such that the maximum equilibrium yield (MEY) varies directly with the value of B_0. Conversely, given the MEY value for a population (plus estimates for W_∞, ω, t_m, f_{max}, α_{max} M, β, t_c), its B_0 value can be derived. This useful property provides a simple means of formally incorporating empirical relationships between population yield and limiting habitat into our population model. It permits such a relationship to be translated into a formula that predicts the value of B_0 appropriate to a particular amount of limiting habitat (Appendix 1).

Payne et al. (1990) (cf. Marshall 1996) extended Christie and Regier's (1988) lake yield (kilograms per hectare) data for lake trout by including long-term, sustained lake yield values from five smaller Ontario lakes. They found that annual lake yield could be accurately predicted from lake area ($r^2 = 0.923$, $p < 0.001$, $n = 20$):

$$(6) \qquad \log_{10}(\text{yield}) = 0.50 + 0.83 \cdot \log_{10}(\text{area})$$

and we incorporated this relationship into our model by translating it (Appendix 1) into a relationship that predicts B_0 from lake area:

$$(7) \qquad B_0 = 84.33 \cdot \text{area}^{-0.76+0.038 \cdot \log_{10}(\text{area})}.$$

This was done using all the relationships developed so far plus the relationship between ω, L_∞, and t_c described below.

Vulnerability (t_c)

We used total angling mortality estimates and catch at age data to determine age-specific vulnerability for the lake trout in 21 lakes. Beginning with the modal age in the catch at age data (assumed to be the age of full vulnerability: $v_i = 1$), we back-calculated the expected catch in the preceding age-class from

$$C_i \approx \frac{v_i}{v_{i+1}} \cdot C_{i+1} \cdot e^{v_i \cdot F + M}.$$

We estimated the values of v_i for each age prior to the modal age by trial and error such that the computed C_i from the above equation most closely corresponded to the observed catch at age. We then used these age-specific vulnerabilities to estimate length at 5, 50, and 95% vulnerability (L_{c5}, L_{c50}, L_{c95}) for each of the 21 populations. We used analysis of covariance on these data, with $\log(L_i, i = c5, c50, c95)$ as dependent variable, $\log(L_\infty)$ and $\log(\omega)$ as covariates, and 5, 50, and 95% vulnerability levels as treatments. All independent variables were statistically significant ($p < 0.01$), with the full covariance model explaining 64% ($p < 0.0001$, $n = 63$) of the variation in observed sizes at the three levels of vul-

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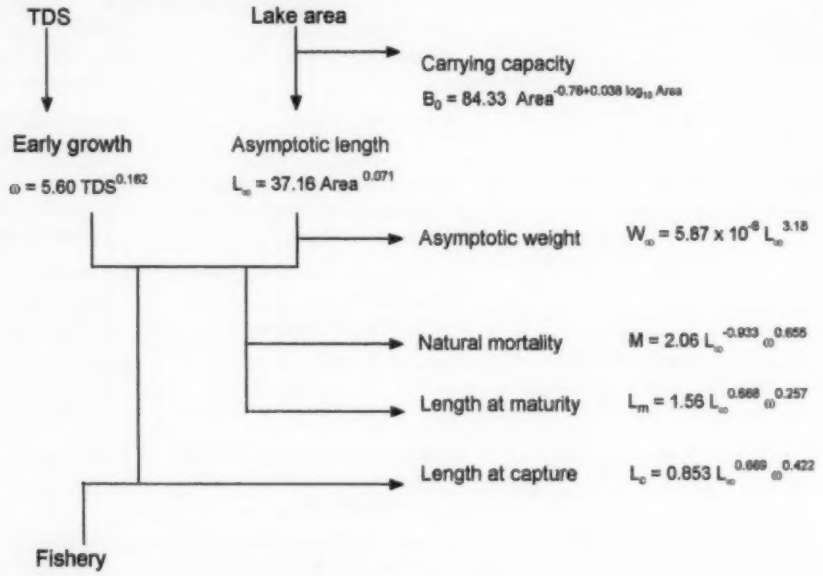
Fig. 2. Schematic diagram showing how eqs. 1–8 can be used to translate information on the area and TDS of a lake into a fully parameterized population model for the lake trout living in that lake.



nerability estimated. This analysis leads to the following equation for predicting size at 50% vulnerability (L_c):

$$(8) \qquad L_c = 0.853 \cdot \omega^{0.421} \cdot L_\infty^{0.669}.$$

Thus, for any lake, L_c can be estimated from ω and L_∞ using eq. 8 and t_c can then be estimated from L_c using the von Bertalanffy growth equation for the lake. The data used in this analysis come from fisheries that were free of regulations on the size of individual fish harvested. Hence, eq. 8 captures the "natural" size preference of fishers for their prey and suggests that this preference varies from population to population depending on the size range of fish available. Using eq. 8, we can estimate t_c from lake size and TDS using the relationships that link these characters to ω and L_∞.

Yield as a function of fishing mortality

We observed that L_∞ varies with lake size, that ω varies with TDS, and that other life history parameters vary in a consistent fashion with these variations in growth. Given this variation, the sensitivity of lake trout populations to exploitation may also vary with lake size and lake fertility. We explored this possibility using a population yield model based on eqs. 1–8 (Fig. 2). Model output showed that lake size has a substantial influence on the level of fishing mortality associated with MEY (F_{MEY}) and on the level of fishing mortality associated with stock extinction (F_{ext}). Yield curves for three lake sizes at a median level of TDS (32 mg·L^{-1}) demonstrate this (Fig. 3*a*; Table 3). For small lakes (100 ha), $F_{MEY} = 0.13$ and $F_{ext} = 0.20$, while for large lakes (10 000 ha), $F_{MEY} = 0.30$ and $F_{ext} = 0.43$. Lake trout populations in large lakes can apparently sustain approximately three times the fishing mortality that populations in small lakes can sustain. The effect of an increase in TDS on sustainable F values is similar to that of an increase in lake size (Fig. 3*b*; Table 3).

The influence of lake size on the yield–F relationship stems from two separate effects. First, asymptotic mass (W_∞) is greater in large lakes (Table 3), and therefore, the egg production of a typical adult is greater. Other factors being equal, this ensures that the typical adult is more efficient at producing offspring and hence that the population can sustain higher total mortality rates. Second, natural mortality rates are lower in large lakes due to the positive association between lake size and adult fish size and the inverse relationship between adult size and natural mortality (eq. 5). Other factors being equal, lower natural mortality ensures lower prereproductive mortality, a longer reproductive lifetime, and thus, higher sustainable fishing mortality rates. Sensitivity analyses have shown that it is the differences in individual growth that contribute most to the differences in sustainable exploitation rates. To summarize, in large lakes, higher total mortality rates are sustainable because egg production per adult is greater, and a larger proportion of this mortality can be allocated to fishing because natural mortality is lower.

The influence of TDS on the yield–F relationship stems from three similar effects. First, mass at first maturity (W_m) is greater and age at first maturity is lower in high-TDS lakes. Hence, adult egg production is greater and exposure to prereproductive mortality is reduced. In addition, the difference between age at first capture and age at first maturity is smaller in high-TDS lakes (Table 3), and this leads to a decrease in prereproductive fishing mortality. All these changes lead to an increase in the range of sustainable total mortality rates and consequently an increase in the range of sustainable fishing mortality rates. The latter increase is diminished but not eliminated by the higher natural mortality

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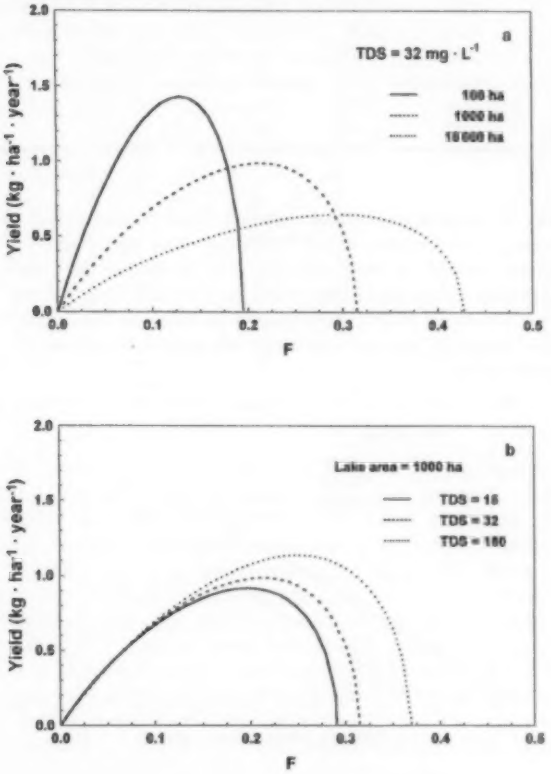
Fig. 3. Predicted relationships between equilibrium yield and fishing mortality for (*a*) lakes with a TDS value of 32 mg·L^{-1} and surfaces areas of 100 ha (solid line), 1000 ha (dashed line), and 10 000 ha (dotted line) and (*b*) lakes with a surface area of 1000 ha and TDS values of 15 mg·L^{-1} (solid line), 32 mg·L^{-1} (dashed line), and 180 mg·L^{-1} (dotted line). Predictions were derived using the lake trout population model, parameterized according to the scheme outlined in Fig. 2.



rates that also accompany the shift in growth pattern characteristic of higher TDS values. The net result of all these changes is higher F_{MEY} and F_{ext} values among populations with higher TDS values.

Fishing mortality as a function of fishing effort and stock abundance

Effort levels on a lake are often used as indicators of exploitation intensity. To consider the effort implications of this model, the relationship between fishing mortality rate (F) and angling effort (E) must be described. Classical fisheries theory (Beverton and Holt 1957; Ricker 1975) assumes that these variables are directly related, $F = qE$, where the catchability coefficient (q) is a constant. Many theoretical (e.g., Paloheimo and Dickie 1964; Condrey 1984) and empirical (e.g., Peterman and Steer 1981; Beaumont et al. 1991) studies have shown that, under a wide variety of circumstances, it is more reasonable to expect that q will vary inversely with the abundance of the exploited population. We constructed three independent tests of the hypothesis that angling q is negatively related to lake trout population abundance (number per hectare).

We examined variation in catchability for two lakes where annual, independent estimates of both catchability and population abundance could be obtained (Fig. 4*a*). Time series data from the lake trout populations in Lake Opeongo and Squeers Lake included annual estimates of effort, yield, population abundance (N, number per hectare), and fishing mortality. For the Opeongo population, F and N estimates were based on catch at age analysis, supplemented with a series of mark–recapture experiments (Shuter et al. 1987) (Appendix 2). For the Squeers population, annual F and N estimates were based on annual mark–recapture experiments (Appendix 2). For each lake, we found a negative correlation between q and N ($p < 0.001$).

We examined variation in catchability among 17 lakes where estimates of average values for both catchability and population abundance (N, number per hectare) could be obtained (Fig. 4*b*). For lakes other than Opeongo and Squeers, we obtained annual estimates of effort intensity by multiplying nominal effort per hectare by the proportion of the catch that was kept. We averaged annual effort and yield values across years to get mean values for annual effort intensity and annual harvest (H, number kept per hectare) for each lake. We used pooled catch at age data to estimate total mortality (Z) (Chapman and Robson 1960; Robson and Chapman 1961) and eq. 5 to estimate natural mortality (M). From these mortality components, we generated lake-specific estimates of F, q, and N:

$$F = Z - M$$

$$q = \frac{F}{E}$$

$$N = \frac{H}{F}.$$

These q and N estimates were also negatively correlated ($p < 0.001$).

All three analyses demonstrate a strong, negative relationship between catchability and population abundance. This finding suggests that density-dependent angler catchability is a common feature of lake trout populations in Ontario.

Yield as a function of fishing effort: influence of density-dependent catchability

Paloheimo and Dickie (1964) were the first to consider the consequences for catchability of a more realistic picture of predation by fishers. Given that fish are distributed in patches (i.e., schools, aggregations) and that fishing involves the search for patches and the capture of fish within patches, they showed that catchability should be modelled by an equation of the form

$$q = \frac{a}{1 + bN}.$$

Linearizing this equation by plotting q^{-1} against N, we see that the relationship defined by pooling the Squeers and Opeongo data sets roughly matches not only the within-lake variation in q observed in the two lakes (Fig. 4*a*), but also

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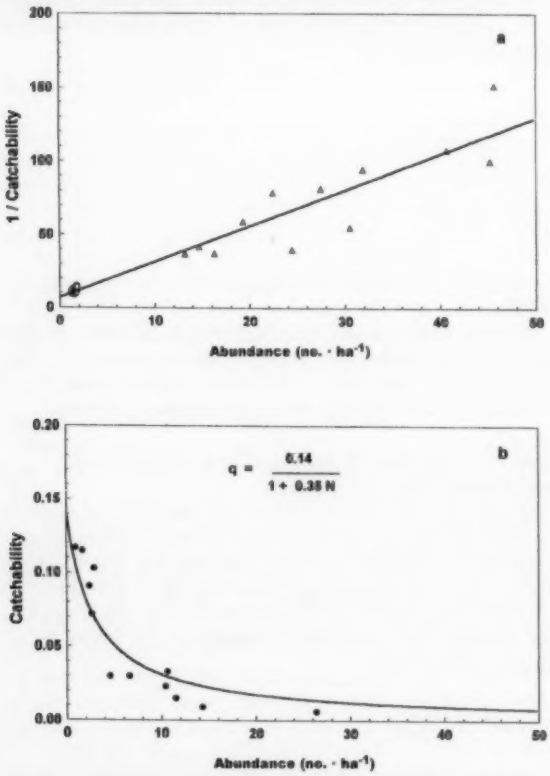
Table 3. Predicted parameter values (W_∞, M, t_m, t_c) and model results (F_{MEY}, F_{ext}, E_{MEY}, E_{safe}) for various lake sizes (100, 1000, 10 000 ha) and levels of TDS (15, 32, 180 mg·L^{-1}).

Lake size (ha)	W_∞ (kg)	TDS (mg·L^{-1})	M (year^{-1})	t_m (years)	t_c (years)	$t_m - t_c$ (years)	F_{MEY} (year^{-1})	F_{ext} (year^{-1})	E_{MEY} (angler-h·ha^{-1})	E_{safe} (angler-h·ha^{-1})
100	1.63	15	0.22	7.8	5.1	2.7	0.12	0.19	6.6	1.3
		32	0.23	7.3	4.9	2.4	0.13	0.20	6.3	1.4
		180	0.28	6.6	4.5	2.1	0.14	0.21	5.4	1.5
1 000	2.74	15	0.18	8.2	5.5	2.7	0.20	0.29	4.2	2.1
		32	0.20	7.7	5.3	2.4	0.21	0.32	4.2	2.3
		180	0.24	6.8	4.8	2.0	0.25	0.37	4.2	2.6
10 000	4.61	15	0.16	8.7	6.0	2.7	0.27	0.39	3.3	2.8
		32	0.17	8.2	5.7	2.5	0.30	0.43	3.4	3.1
		180	0.21	7.1	5.2	1.9	0.37	0.53	3.9	3.8

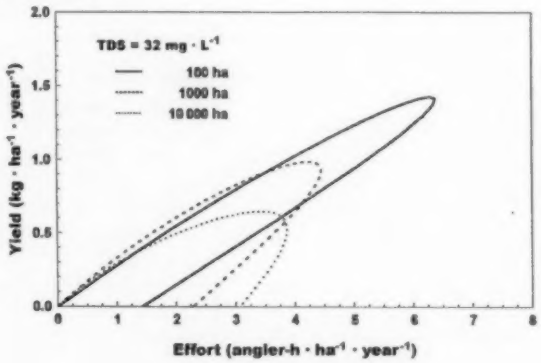
Note: W_∞ is shown only once for each lake size because it is not affected by TDS.

Fig. 4. (*a*) Observed relationship between the inverse of catchability and population abundance for the lake trout populations of Lake Opeongo (circles) and Squeers Lake (triangles). The line is the regression line derived from all the data points. Note that the fitted line closely matches the trend evident in the Squeers Lake data alone while also passing through the centres of the two clusters of data. (*b*) Fitted relationship (from Fig. 4*a*) between catchability (q) and abundance (N) compared with observed values for average catchability and abundance for 12 Ontario lake trout lakes.



the among-lake variation in q observed for the 12 lakes with mean densities > 1 individual·ha^{-1} (Fig. 4*b*). The relationship does, however, underestimate q values for the three low-density (<0.5 individual·ha^{-1}) lakes in our data set (predicted q = 0.15, mean observed q = 0.31). Given the many factors that can influence catchability, it is perhaps surprising that a single relationship is capable of describing so much of the joint variation observed among q and N values.

Fig. 5. Predicted relationships between equilibrium yield and fishing effort for lakes with a TDS value of 32 mg·L^{-1} and surface areas of 100 ha (solid line), 1000 ha (dashed line), and 10 000 ha (dotted line). Predictions were derived using the lake trout population model, parameterized according to Fig. 2, plus the relationship between catchability and population abundance given in Fig. 4.



We used this relationship to reexpress the yield–F curves in Fig. 3 in terms of yield–E curves: we substituted the equilibrium population abundance at each F value (Appendix 1) into the following equation to calculate the appropriate equilibrium effort value:

$$E = \frac{F}{\frac{0.14}{1 + 0.35N}}.$$

This leads to a set of unusual yield–E curves (Fig. 5). As effort increases toward E_{MEY}, equilibrium yield rises almost linearly to a maximum (MEY). Small increases in effort beyond E_{MEY} are unstable and lead to stock collapse if maintained. These curves are the product of the strong interaction between population abundance and sustainable effort levels explained below (Fig. 6).

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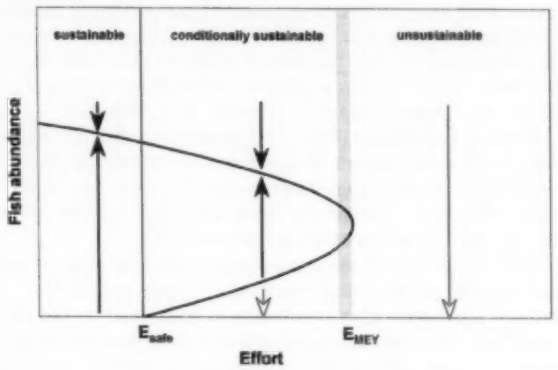
Fig. 6. Schematic diagram illustrating changes in equilibrium population abundance (N) with changes in fishing effort (E). If $E < E_{safe}$, there is a single equilibrium value for N and that equilibrium value is stable. If N lies between E_{safe} and the maximum equilibrium value for E, there are two equilibrium values for N, a higher one that is stable and a lower one that is unstable. If N is greater than the maximum equilibrium E, there are no equilibrium values for N. The open and solid arrows show how N will change if it does not lie on one of the equilibrium curves.



(*i*) There is a critical effort level, E_{safe}, below which all effort values are unconditionally sustainable; for each effort level in this interval, population abundance will move toward a stable, equilibrium value that is well above zero.

(*ii*) Values of E in the interval (E_{safe}, E_{MEY}) are conditionally sustainable; for each effort level in this interval, there is a critical value for population abundance, N^*, such that (*a*) if $N > N^*$ and the effort level is maintained, the population will move toward a stable equilibrium value that is well above zero and (*b*) if $N < N^*$ and the effort level is maintained, the population will collapse.

(*iii*) Values of $E > E_{MEY}$ are unsustainable; if such effort levels are maintained, the population will collapse.

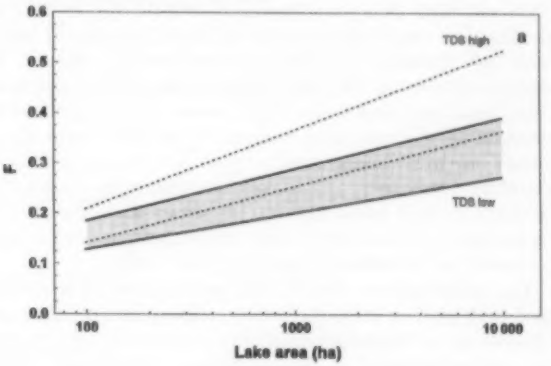
This pattern of behaviour has the important consequence that, when effort is at unsustainable levels ($N < N^*$, given E), hesitation in implementing effort reductions will ensure that larger reductions are necessary to permit stock recovery. This occurs because when $N < N^*$, N will decline and as N declines, the effort level that it can sustain also declines. The longer this decline is allowed to continue, the greater the effort reduction required to stabilize N and permit it to increase. In the absence of information on stock abundance, E must be reduced below E_{safe} to ensure stock recovery. The highly stochastic nature of many real systems greatly augments the danger of inadvertently entering a region of unsustainable effort levels. Once this happens, the dynamics outlined above will take over, leading to the all too familiar situation of a collapsed population and a closed fishery.

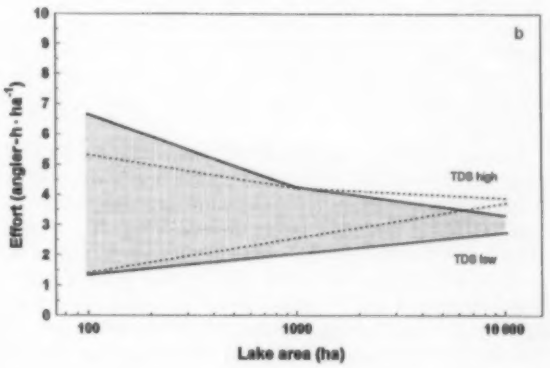
Discussion

Managing Ontario lake trout populations: strategic considerations

We had two primary objectives in this study. First, we identified predictable differences in the biology of Ontario lake trout populations and in the character of Ontario lake trout angling fisheries. Second, we demonstrated the effects of such differences on the sensitivity of individual populations to overexploitation. Some of our findings have significant implications, at the strategic level, for exploitation management in the province.

Fig. 7. (*a*) Changes in predicted benchmark mortality values (F_{MEY}, F_{ext}) with changes in lake size and TDS. The elevated pair of dashed lines represents F_{ext} (top line) and F_{MEY} (bottom line) values for lakes with a TDS of 180 mg·L^{-1}. The pair of solid lines represents F_{ext} (top line) and F_{MEY} (bottom line) values for lakes with a TDS of 15 mg·L^{-1}. (*b*) Changes in predicted benchmark fishing effort (E_{safe}, E_{MEY}) levels with changes in lake size and TDS. The pair of dashed lines represents E_{MEY} (top line) and E_{safe} (bottom line) values for lakes with a TDS of 180 mg·L^{-1}. The pair of solid lines represents E_{MEY} (top line) and E_{safe} (bottom line) values for lakes with a TDS of 15 mg·L^{-1}.





Our analysis shows that small lakes with low TDS values are quite sensitive to overexploitation and hence require more stringent exploitation controls than large lakes with high TDS values. Both sustainable fishing mortality levels and sustainable effort levels vary significantly with lake area and with TDS (Table 3; Fig. 7). As lake size decreases or as TDS decreases, values for F_{MEY} and F_{ext} decline and converge, while values for E_{MEY} and E_{safe} diverge. These shifts indicate that large, high-TDS lakes are least sensitive to exploitation, while small, low-TDS lakes are most sensitive: as

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F_{ext} approaches F_{MEY}, small variations in F around F_{MEY} are more likely to exceed F_{ext} and hence lead to stock collapse; similarly, as E_{safe} diverges further from E_{MEY}, larger reductions in fishing effort will be required to ensure stock recovery, once collapse has begun.

Values for E_{safe} and E_{MEY} vary in a complex fashion with both lake area and TDS. For any TDS value, E_{MEY} decreases with lake area, while E_{safe} increases (Fig. 7*b*). For small lakes, E_{MEY} decreases with TDS, while E_{safe} remains essentially unchanged. For large lakes, E_{safe} and E_{MEY} increase and converge with TDS. This interactive effect arises because both growth rate and size at maturity increase with TDS and these changes permit numerical density at MEY to decline. For small lakes, numerical density is high to begin with and, therefore, catchability is particularly sensitive to density differences. For such lakes, the lower numerical densities associated with higher TDS values lead to increases in catchability that are large enoough to produce lower E_{MEY} values. In large lakes, density is low to begin with and, therefore, catchability is relatively insensitive to density differences. In such lakes, higher TDS values are accompanied by increases in benchmark effort levels that roughly parallel increases in benchmark fishing mortality rates.

Our analysis shows that the catchability coefficient associated with angling effort is strongly dependent on lake trout abundance, and therefore, catch per unit effort (CUE) figures are not proportional to fish abundance. Preliminary work with a dynamic version of our model shows that the density dependence of catchability is strong enough to permit maintenance of unsustainable effort levels for extended periods before a detectable decline in CUE occurs. These results demonstrate that angler CUE cannot be used to accurately assess the status of lake trout populations subjected to angling fisheries.

Another important aspect of the interaction between angling catchability and population abundance is the fact that sustainable effort levels decrease with lake size, while sustainable fishing mortality levels increase (Fig. 7). Observed effort levels for Ontario lake trout lakes tend to follow this trend; however, they are often greater than predicted E_{MEY} values: predicted E_{MEY} values lie in the range from 3 to 7 angler-h·ha^{-1}·year^{-1}. In the Goddard et al. (1987) study of 80 Ontario lake trout angling fisheries, about half the recorded effort values (median effort = 7.7 angler-h·ha^{-1}) exceeded this range. Thus, existing angling effort levels appear to be well in excess of sustainable limits on many of Ontario's lake trout lakes. The effects of overexploitation are obvious in the southern part of the province, where high effort levels have existed for many years, severe reductions in angling CUE values have occurred, and these reductions have led to both new regulations and an increased demand for stocking. Effects are less obvious in the north, where lakes have a shorter history of exploitation.

Critical elements in regional management models for fish populations

Density-dependent catchability and effort management

Classical fisheries theory (Beverton and Holt 1957; Ricker 1975) and common management practice begin with the assumption of a constant catchability coefficient. Strictly speaking, this assumption is only valid if the movements of either predator (the fisher) or prey (the fish) are purely random. While some fisheries may approximate this ideal condition, many do not. The behavioural repertoire of many fish species is as complex as that of any of the vertebrates, with habitat preference, habitat defense, and schooling commonly exhibited. These very nonrandom movement habits of the prey are complemented by the ability of typical fishers to find and efficiently exploit concentrations of fish.

Early theoretical work by Paloheimo and Dickie (1964) showed that more realistic pictures of the predation process in fisheries require that catchability be represented as a negative function of fish population density. This early result has been reinforced by a long list of additional theoretical work, plus many empirical studies (e.g., MacCall 1976; Hutchings and Myers 1994). The theoretical studies suggest that, given the character of real fisheries, density-dependent catchability should often be expected. The empirical studies show that, when looked for, such an effect is more often present than absent (Lester and Shuter 1993).

The weight of this evidence suggests that the appropriate null hypothesis for most situations is that catchability is density dependent. This conclusion is reinforced by the precautionary principle that effort management strategies predicated on a false assumption of constant catchability can lead directly to stock collapse (e.g., Fig. 6).

Yield, carrying capacity, and habitat quantity

Our yield model explicitly identifies the linkages between the amount of habitat available to a population and the sustainable yields that the population can generate. We do this using a very simple version of the approach to habitat modelling discussed by Shuter (1990) and Minns et al. (1996). This approach permits easy scaling of model output to match empirical relationships that predict sustainable yields from indices of available habitat. In our lake trout analysis, this was done by making B_0 a function of lake area so that model-generated MEY values would match values predicted from an empirical relationship linking long-term sustained yield to lake area. Such empirical relationships have been demonstrated for other species. Christie and Regier (1988) found strong links between sustainable yield and amount of thermally suitable habitat for walleye (*Stizostedion vitreum*), northern pike (*Esox lucius*), and lake whitefish (*Coregonus clupeaformis*). Thus, it seems likely that habitat-based predictors for MEY can be developed for other species. Incorporating such relationships into yield models greatly extends model usefulness by allowing explicit consideration of the effects of habitat loss on sustainable yield.

Predictability of interpopulation differences in life history characters

Our work on lake trout has shown that interpopulation differences among life history characters can be large enough to significantly affect sustainable exploitation rates. Within the relatively narrow geographic confines of Ontario, lake trout populations exhibit a twofold variation in both ω and L_∞ and a fourfold variation in M (Tables 1 and 2). Given this degree of variation in life history parameters, we can infer up to a fourfold variation in benchmark (F_{MEY}, F_{ext}) exploitation rates. This degree of variation is not unique to lake

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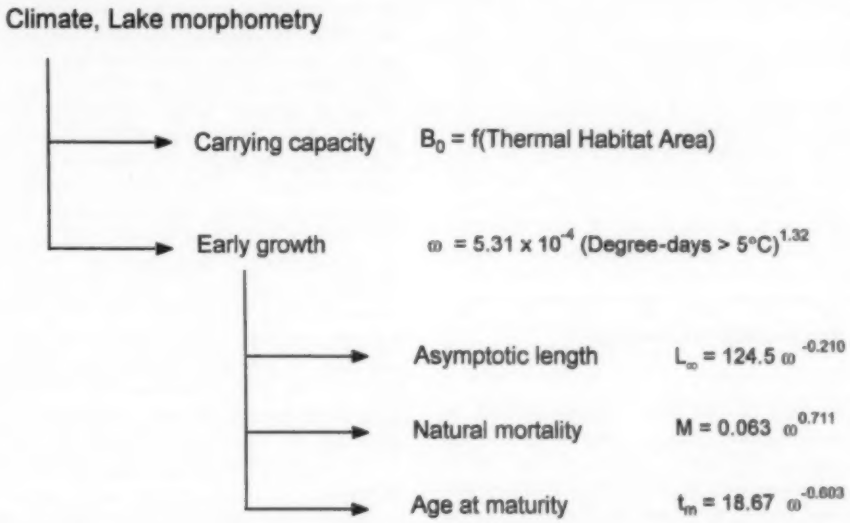
Fig. 8. Empirical relationships linking environmental variables to carrying capacity and life history parameters for walleye populations. The carrying capacity relationship refers to the finding of Christie and Regier (1988) that sustainable yields from walleye populations were tightly correlated with a measure of the annual average amount of habitat thermally suitable for walleye (i.e., their "thermal habitat area"). The life history relationships were derived from regression analysis of walleye life history data reported in Beverton (1987). Natural mortality rates were estimated from longevity values using a relationship between mortality and longevity reported by Hoenig et al. (1987).



trout. Life history data from walleye populations in 13 lakes spanning the much wider geographical range from Ontario to Texas (Beverton 1987, based on data from Colby et al. 1979 and Colby and Nepszy 1981) exhibit a twofold range in L_∞, a 10-fold range in ω, and a fourfold range in M. Given the potential effects of such variation on sustainable exploitation rates, it is obvious that exploitation could be more effectively managed if this variation could be predicted and used to identify geographical regions and (or) categories of water bodies that require different regulations.

In general, interpopulation variation in demographic parameters can arise from two sources: variation in the abiotic characteristics of the habitats occupied by the populations or variation in the biota that share those habitats. The walleye data mentioned above suggest an abiotic linkage: life history parameters are strongly correlated with an index of environmental temperature, and with each other (Fig. 8). This set of demographic–environmental linkages is quite similar to the set that characterizes Ontario lake trout populations (Fig. 2): growth (ω) is predictable from environmental conditions, and maturity and mortality are predictable from growth.

For lake trout, the effects of TDS and lake area on growth probably reflect differences in associated biota rather than differences in abiotic conditions. Lake trout are strongly stenothermal and tend only to be found in lakes (and habitats within lakes) that provide abiotic conditions that are well suited to their requirements (e.g., low temperatures, high dissolved oxygen levels). Higher TDS levels are associated with higher zooplankton productivity, and zooplankton are the primary food for lake trout during their first few years of life. Hence, the TDS effect on ω may reflect a relatively simple link between food availability and growth. The lake area effect on L_∞ is a bit more difficult to interpret. However, there is considerable evidence that lake trout lakes with greater community complexity (most notably the presence of a broad size range of abundant prey) tend to produce populations with larger asymptotic sizes (Martin 1966; Matuszek et al. 1990). Our data are consistent with this trend. For the 54 populations that we examined, the log–log correlation between L_∞ and lake area is matched by similar log–log correlations between L_∞ and species richness (r^2 = 0.39, $p < 0.001$) and between species richness and lake area (r^2 = 0.53, $p < 0.001$). Also, the fact that the growth differences among populations become more apparent as fish reach older ages is consistent with the expectation that in larger lakes, greater fish species richness will provide a wide prey size range and hence promote increased growth among larger, piscivorous lake trout (Kerr and Martin 1970; Kerr 1971; Roff 1986).

Both of our examples exhibit strong intercorrelations among life history parameters. This is consistent with many empirical and theoretical studies that support the existence of significant covariation among the primary life history parameters of fish, most notably those that describe growth (ω, L_∞), sexual maturity (L_m, t_m) (Roff 1992, Charnov 1993), and natural mortality (e.g., Beverton 1987; Charnov and Berrigan 1991; Roff 1991). Qualitatively similar relationships seem to describe both inter- and intra-specific variation in these characters (e.g., Pauly 1980; Roff 1992; Charnov 1993).

Life history theory (Roff 1992; Charnov 1993) seeks to explain such intercorrelations by suggesting that optimization of lifetime reproductive output requires that variation in growth rate produce changes in size at first maturity and that these changes will, in turn, affect postreproductive mortality. Similarly, variation in mortality rate should produce changes

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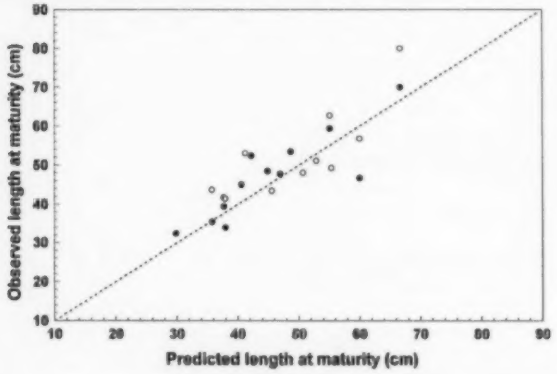
Fig. 9. Observed and predicted values for size at maturity. Observed values represent the lengths at which 50% (solid circles) and 95% (open circles) of females are mature; predicted values were derived from von Bertalanffy growth parameters using an equation (eq. 9) developed by Roff (1986).



in age and size of maturity and these changes should, in turn, affect the overall growth pattern of the animal. Much of the covariation among life history characters observed in our examples is consistent with Roff's (1984, 1986) model for predicting optimal size at first reproduction. Roff found that, for a wide range of circumstances, the size at first reproduction that maximizes lifetime reproductive output is

$$(9) \qquad L_m = L_\infty \cdot \left(\frac{3 \cdot \omega}{3 \cdot \omega + M \cdot L_\infty} \right)$$

and the corresponding age at first reproduction is

$$(10) \qquad t_m = \frac{L_\infty}{\omega} \cdot \log_e \left(\frac{3 \cdot \omega}{L_\infty \cdot M} + 1 \right)$$

We used these equations to predict L_m and t_m values from our estimates for L_∞, ω, and M. We found good correspondence (Fig. 9) between predicted L_m values and our observed values for L_{m50} and L_{m95} and reasonable correspondence between estimated and predicted t_m values ($R^2 = 0.334$, $p < 0.003$, $n = 23$, relationship between estimated and predicted values not significantly different from the 1:1 line).

Equations 9 and 10 predict that growth rate changes at different points in the life cycle can have different effects on life history parameters: an increase in L_∞ will cause both L_m and t_m to increase, while an increase in ω will cause L_m to increase and t_m to decrease. These predictions are confirmed by our lake trout data. Increases in lake size are accompanied by parallel increases in both L_m and t_m; increases in TDS are accompanied by increases in L_m and decreases in t_m. These effects have been observed in earlier studies. Matuszek et al. (1990) observed an increase in both size and age at maturity in the Lake Opeongo lake trout population after the introduction of cisco (*Coregonus artedi*) produced increased growth among lake trout > 35 cm in length. Trippel (1993) and Trippel and Beamish (1993), in a comparative study of three low-TDS and three high-TDS lakes, found higher early growth rates, higher sizes at maturity, and lower ages at maturity in the three high-TDS lakes.

Thus, fish species that typically undergo ontogenetic shifts in diet (e.g., lake trout, walleye) may respond to a growth rate increase with either an increase or a decrease in age at maturity, depending on where in the life cycle the growth rate change occurs (Hutchings 1993). Fish that do not exhibit ontogenetic diet shifts are more likely to exhibit growth rate changes throughout the life cycle and hence are more likely to decrease age of maturity in response to an increase in growth. These conclusions are a simple consequence of the basic trade-off that underlies optimization for age of maturity in fish: the benefits gained by delaying maturity to a later age (and hence, growing to a larger size) are increased fecundity and decreased cost of reproduction, while the cost incurred is a decrease in the overall probability of surviving to that later age. An increase in potential growth for age groups near the current age of maturity would increase the benefit of growing older without affecting the cost, and hence would move both the optimum age and size of maturity upward. An increase in growth rate early in life would decrease the benefit in growing older without decreasing the cost, and hence would move the optimum age of maturity downward and size at maturity upward, as the increase in growth compensates for the decrease in age.

Our results suggest that the search for useful environmental correlates of life history characters should focus on those factors most likely to influence the lifetime growth pattern of the species being studied. Our results support the prediction of life history theory that a common consequence of natural selection for reproductive efficiency is a high degree of intercorrelation among life history parameters. Thus, once such a correlation pattern has been identified in a representative sample of populations from a region, that pattern can be used with some confidence to forecast behaviour of other populations in that region.

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Appendix 1. Derivation of equilibrium yield equation

Our model combines yield per recruit and stock–recruitment functions to construct yield curves relating equilibrium yield to fishing mortality, following methods described by Shepherd (1982). We will focus on the situation where the age of first capture by the fishery (t_c) is less than or equal to the age of maturity (t_m).

First, we note that yield (Y) equals yield per recruit (YPR) times number of recruits (R):

$$\text{(A1)} \quad Y = \text{YPR} \cdot R$$

and that the number of recruits equals female spawner biomass (S) divided by female spawner biomass per recruit (SPR):

$$\text{(A2)} \quad R = \frac{S}{\text{SPR}}.$$

Therefore, yield can be expressed as

$$\text{(A3)} \quad Y = \text{YPR} \cdot \frac{S}{\text{SPR}}.$$

Given any stock–recruitment relationship, spawner biomass can be expressed as a function of SPR. This is illustrated using Shepherd's generic model which accommodates various functional forms proposed by Ricker (1954, 1975) and Beverton and Holt (1957):

$$\text{(A4)} \quad R = \frac{\alpha_{\max} \cdot f_{\max} \cdot S}{1 + \left(\frac{S}{B_0}\right)^{\beta}}$$

where $\alpha_{\max}$ is survival from conception to recruitment, $f_{\max}$ is fecundity, and B_0 and β together control the rate at which the number of recruits per unit spawner biomass declines with increases in spawner biomass. Because SPR is defined as S/R, eq. A4 can also be expressed in the form

$$\text{(A5)} \quad S = B_0 \cdot \left(\alpha_{\max} \cdot f_{\max} \cdot \text{SPR} - 1\right)^{\frac{1}{\beta}}.$$

Substituting for S in eq. A3 gives

$$\text{(A6)} \quad Y = \frac{\text{YPR}}{\text{SPR}} \cdot B_0 \cdot \left(\alpha_{\max} \cdot f_{\max} \cdot \text{SPR} - 1\right)^{\frac{1}{\beta}}.$$

The variables in this equation, YPR and SPR, are in fact functions that depend on life history parameters (i.e., growth, age at maturity, and natural mortality) and fishery parameters (i.e., initial age of capture and fishing mortality). A function for YPR is available from Beverton and Holt (1957):

$$\text{(A7)} \quad \text{YPR} = F \cdot e^{-M \cdot (t_c - t_r)} \cdot W_\infty \times \left(\frac{1}{Z} - \frac{3 \cdot e^{-K \cdot t_c}}{Z + K} + \frac{3 \cdot e^{-2 \cdot K \cdot t_c}}{Z + 2 \cdot K} - \frac{e^{-3 \cdot K \cdot t_c}}{Z + 3 \cdot K}\right)$$

where F is fishing mortality, M is natural mortality, t_c is initial age of capture, t_r is the age of a recruit (whose abundance is indicated by R in previous equations), W_∞ is asymptotic mass, K is the von Bertalanffy growth rate parameter, and Z is total mortality ($= M + F$). To simplify the notation, the bracketed expression in eq. A7 will be referred to as function $g(t)$ where

$$\text{(A8)} \quad g(t) = \left(\frac{1}{Z} - \frac{3 \cdot e^{-K \cdot t}}{Z + K} + \frac{3 \cdot e^{-2 \cdot K \cdot t}}{Z + 2 \cdot K} - \frac{e^{-3 \cdot K \cdot t}}{Z + 3 \cdot K}\right)$$

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An equation for SPR can be derived from the YPR function given that (*i*) catchable biomass (*B*) equals yield divided by fishing mortality:

$$(A9) \quad B = \frac{Y}{F}$$

and (*ii*) spawner biomass equals catchable biomass times the proportion (by mass) of mature fish in the catchable segment of the population (p_m) times the proportion (by mass) of females (i.e., 0.5):

$$(A10) \quad S = 0.5 \cdot B \cdot p_m.$$

Substitute eq. A9 into eq. A10 and divide both sides by *R* to obtain

$$(A11) \quad \mathrm{SPR} = \frac{\mathrm{YPR} \cdot p_m}{2 \cdot F}.$$

To obtain an equation for p_m, we note that the proportion of mature fish in the catchable segment equals the proportion of mature fish in the catch. An expression for the latter is available by an extension of eq. A7 which gives the yield provided by fish that are aged *t* or older ($t > t_c$) as a function of the number of fish that survive to age *t* (N_t):

$$(A12) \quad Y_t = F \cdot N_t \cdot W_\infty \cdot g(t).$$

Since

$$(A13) \quad p_m = \frac{Y_{t_m}}{Y_{t_c}}$$

it follows that, when $t_c \leq t_m$:

$$(A14) \quad p_m = \frac{N_{t_m} \cdot g(t_m)}{N_{t_c} \cdot g(t_c)}$$

Given that the number of fish surviving to maturity is less than or equal to the number at initial age of capture:

$$(A15) \quad N_{t_m} = N_{t_c} \cdot e^{-Z \cdot (t_m - t_c)}$$

and therefore:

$$(A16) \quad p_m = e^{-Z \cdot (t_m - t_c)} \cdot \frac{g(t_m)}{g(t_c)}$$

and

$$(A17) \quad \mathrm{SPR} = \frac{\mathrm{YPR}}{2 \cdot F} \cdot e^{-Z \cdot (t_m - t_c)} \cdot \frac{g(t_m)}{g(t_c)}$$

Substitute the expression in eq. A7 for YPR to get

$$(A18) \quad \mathrm{SPR} = 0.5 \cdot W_\infty \cdot e^{-M \cdot (t_c - t_r)} \cdot e^{-Z \cdot (t_m - t_c)} \cdot g(t_m)$$

An explicit formulation for yield is then obtained by substituting for YPR and SPR in eq. A6:

$$(A19) \quad Y = \frac{2 \cdot B_0 \cdot F \cdot g(t_c)}{e^{-Z \cdot (t_m - t_c)} \cdot g(t_m)} \times (0.5 \cdot \alpha_{max} \cdot f_{max} \cdot W_\infty \cdot e^{-M \cdot (t_c - t_r)} \cdot e^{-Z \cdot (t_m - t_c)} \cdot g(t_m) - 1)^{\frac{1}{\beta}}$$

Thus:

$$(A20) \quad Y = B_0 \cdot f(M, K, W_\infty, t_m, \alpha_{max}, f_{max}, \beta, t_c, F)$$

$$(A21) \quad = B_0 \cdot f^\circ(F)$$

given all other parameters held constant.

Now, given biologically reasonable values for (M, K, W_∞, t_m, α, f, β, t_c), f°(F) has a single maximum at F_{MEY} and this equals the maximum equilibrium yield (MEY). Therefore:

$$(A22) \quad Y_{MEY} = B_0 \cdot f^\circ(F_{MEY})$$

and

$$(A23) \quad B_0 = \frac{Y_{MEY}}{f^\circ(F_{MEY})}$$

Therefore, if Y_{MEY} is an empirical function (g) of the amount of limiting habitat (hab) and if $f^\circ(F_{MEY})$ is known for each value of hab, then

$$(A24) \quad B_0 = \frac{g(\mathrm{hab})}{f^\circ(F_{MEY})} = g^\circ(\mathrm{hab})$$

Appendix 2. Estimating lake trout density on Squeers Lake and Lake Opeongo

Mark–recapture studies have been conducted since 1985 on Squeers Lake. We used the Peterson–Chapman method to estimate initial abundance (N) of the exploitable stock in each year. Since the fishery is restricted to a 10-day period in March each year, we assumed negligible natural mortality during this period and calculated fishing mortality rate (F) as

$$F = -\log_e\left(1 - \frac{H}{N}\right)$$

and average abundance ($\bar{N}$) as

$$\bar{N} = \frac{H}{F}$$

where H is the annual harvest.

The Lake Opeongo fishery has been monitored since 1937, and estimates of population size and fishing mortality were calculated as described in Shuter et al. (1987) using catch at age analysis and periodic independent assessments with mark–recapture studies. We used only the more recent years (post-1967) in our analysis because the character of the fishery has been relatively stable since then. The fishery operates over a 4-month period (May–August), so we assumed that natural mortality during the fishing period was one third of the annual estimate ($M = 0.11$) and $\bar{N}$ calculated as

$$\bar{N} = N \cdot \frac{(1 - \exp(-F + 0.33 \cdot M)}{(F + 0.33 \cdot M)}.$$

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Consideration of ocean conditions in the management of salmon

Gustavo A. Bisbal and Willis E. McConnaha

Abstract: An ecologically based conceptual foundation is presented as a way to incorporate environmental variability in salmon management. The argument addresses the problem of accommodating marine environment variability through actions in freshwater. We argue for an approach that considers marine and freshwater environments as integral components of a larger salmonid ecosystem. This contrasts with previous propositions that either relied heavily on technological fixes in the freshwater environment or questioned the value of any recovery effort designed to withstand overwhelming ocean forces. Salmon management requires a more holistic approach incorporating modern understanding of the salmonid ecosystem and its variability. We suggest two strategies. The first calls for deliberate improvement of estuarine and nearshore ocean conditions through regulation of upstream flows, river operations, hatchery production, and other actions. The second calls for improving the resilience of salmon to a variable environment. This requires relaxing anthropogenic factors that hinder the natural range of salmon life history diversity within and between populations, a survival mechanism that evolved in response to changing conditions. These strategies recognize that although the ocean primarily determines overall salmon abundance, management actions, particularly in freshwater, are critical to the ability of salmon to cope with a variable ocean environment.

Résumé : Nous présentons un exposé écologique théorique visant à appliquer le principe de la variabilité environnementale à la gestion du saumon. Nous préconisons l'application de mesures en eau douce pour prendre en compte la variabilité du milieu marin et nous soutenons une approche qui considère que les milieux marins et dulcicoles sont des composants faisant partie intégrante de l'écosystème plus vaste des salmonidés. Cette position est très différente des propositions antérieures qui avaient recours à d'importantes interventions technologiques dans les milieux dulcicoles ou qui mettaient en doute la valeur de tout effort de rétablissement visant à résister à la puissance des forces océaniques. La gestion du saumon nécessite une approche plus holistique qui intègre les connaissances modernes sur l'écosystème des salmonidés et sa variabilité. Nous suggérons deux stratégies : la première préconise une amélioration délibérée des conditions océaniques estuariennes et côtières par la régulation des débits d'amont, des activités riveraines, de la production des écloseries et d'autres activités, et la deuxième, l'amélioration de la faculté d'adaptation du saumon à un environnement variable, ce qui nécessite l'atténuation des facteurs anthropiques qui limitent la gamme naturelle de la diversité du cycle biologique des saumons, tant à l'intérieur des populations qu'entre elles; cette diversité est un mécanisme de survie qui a évolué en réponse à des conditions changeantes. Ces stratégies sont fondées sur la constatation du fait que, bien que l'abondance du saumon, dans son ensemble, soit surtout déterminée par les conditions océaniques, des mesures de gestion, notamment dans les milieux d'eau douce, sont cruciales pour que le saumon puisse s'adapter à un milieu océanique variable.

[Traduit par la Rédaction]

Introduction

On September 12, 1996, the U.S. Congress enacted the first and only amendment to the Northwest Power Act of 1980 (Northwest Power Planning and Conservation Act, section (4)(h)(10)(D). 1996. 16 United States Code, section 839b (h)(10)(D)). The original federal law authorized the states of Idaho, Montana, Oregon, and Washington to form the Northwest Power Planning Council (the Council) and called for the Council to develop the Columbia River Basin Fish and Wildlife Program. The first version of the program was released in 1982 with its most recent revision in 1994 (NPPC 1994). The program addresses the restoration of fish and wildlife affected by hydroelectric development in the Columbia River Basin. The recent amendment directed the Council to "...consider the impact of ocean conditions on fish and wildlife populations..." during the implementation of its program. Consideration of ocean conditions has its most direct impact on anadromous fish populations, such as salmon and steelhead. In this paper, we will suggest how the consideration of ocean conditions can be incorporated into salmon management, especially as it relates to the Council's mission in the Columbia River.

During their anadromous life cycle, Pacific salmon utilize riverine and stream environments, but spend most of their lives at sea. Management of salmon populations, however, has typically stressed manipulations of elements in the freshwater phase of this cycle. In general, the forces and pro-

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cesses affecting salmon in the marine environment have been largely ignored (Hare and Francis 1995). The ocean has been regarded as a virtually inexhaustible pasture for juvenile fish produced through actions taken in the freshwater environment (Pearcy 1992). Hatcheries and other approaches in freshwater have been implemented to compensate for habitat deterioration, to increase numbers of fish produced, and to smooth out natural fluctuations in abundance (Lichatowich 1997). Failure to maintain adult salmon abundance through these controlled actions indicates that ocean conditions are, in fact, highly variable and major determinants of the fate of entire fish runs. For some, the pendulum has shifted to the conclusion that management actions during the freshwater phase of salmon life are relatively futile in the face of the mortality and variability experienced by salmon during the marine phase of their life.

In this paper, we suggest the need for a more holistic view of the salmon ecosystem that encourages a new perspective on the importance of ocean conditions and their inclusion in the management of salmon. Both ocean and freshwater conditions, and their variability, are now accepted as integral components of the salmon ecosystem (NRC 1996; Williams et al. 1996). We now have a greater appreciation for the impact of the ocean on salmon abundance and the degree of variation in the marine environment. Throughout their life cycle, salmon negotiate environmental variability by having a broad array of biological characteristics within and between populations. This diversity provides different options for salmon to cope with the mortality they experience during their life cycle in general, including their marine residence. Because management actions taken in freshwater can restrict biological diversity, consideration of ocean conditions in the management of anadromous fish will require relaxation of those constraints that lead to environmental and biological simplification.

Variability in the ocean environment

Many factors can potentially affect the growth and survival of salmon at sea. Physical events such as extreme environmental conditions and biological interactions such as competition and predation can result in substantial variability in salmon recruitment (Pearcy 1992). In addition to natural fluctuations of environmental conditions, salmon encounter elements introduced by humans. These impacts are particularly acute in the estuary and river plume. Human actions can affect estuarine and coastal ocean conditions through pollution, river operations, hatcheries, harvest, or habitat changes. The response of salmon to such environmental challenges differs according to important biological features including life stage, body size, age, growth rate, and previous exposure to specific conditions. What follows provides some insight into the sources of variability that challenge salmon as they enter the ocean.

For the purpose of this paper, the ocean environment is defined relative to the ocean migration of Columbia River salmon. This includes the Columbia River estuary, the Columbia River plume, and the nearshore and offshore areas of the northeastern Pacific extending from northern California to the Gulf of Alaska.

Estuary

The Columbia River estuary represents the only "access gate" for juvenile salmonids migrating downriver and out to the ocean and for returning adults. Although the estuary is an important nursery area for salmon and steelhead smolts, the actual duration of estuarine residence varies with different life histories (Healey 1982). Some studies suggest that juvenile salmonids quickly move through the estuary and plume into the nearshore ocean. However, other studies indicate that the transition may be longer because these habitats provide conditions that reduce predation, improve growth, and reduce physiological stress of many salmonids (Pearcy 1992). Variability in biological and physical components of estuaries can be driven by tidal fluctuations in the short term, by seasonal changes in freshwater flows, by long-term alterations in hydrology and climate, or by changes in land use within the drainage basin (Wolfe and Kjerfve 1986).

The estuary also is affected by many actions that occur upstream (Simenstad et al. 1992). Human activities have caused major modifications of freshwater runoff, sediment transport, land use, vegetation cover, and fluxes of energy and materials in the estuarine system (Sherwood et al. 1990). Construction of over 400 dams in the Columbia River for hydroelectric generation, irrigation, or water storage has impaired fish runs and severely damaged the estuarine and nearshore habitat of salmon (Weitkamp 1994). One of the most important effects of the development of the hydroelectric system was the alteration of the natural water cycle, which reduced spring flows to about 50% of historic levels (Simenstad et al. 1992). The annual contribution of woody debris, sediments, and nutrients to the estuary also has been reduced by half since the dams were put in place (Sherwood et al. 1990; Simenstad et al. 1992). Dredging, diking, and filling altered, reduced, or eliminated many estuarine habitats such as swamps, marshes, and tidal flats (Sherwood et al. 1990).

Upstream development and operation of the hydroelectric system resulted in biological consequences in the estuary as well. Seasonal and historic variability in temperatures, salinities, and quality of habitats may strongly influence food distribution, community structure, and the biological processes of the migratory fauna (Sherwood et al. 1990). Changes in flows and habitat have altered the species composition and the food web, replacing macrodetritus originating from marsh and swamp vegetations with microdetritus from upstream phytoplankton production. Shortages in food availability may be aggravated by the temporal and spatial overlap of juvenile salmonids from different origins. Potential competition for food may occur when large releases of hatchery smolts enter the estuary. These interactions may turn the estuary into a less appealing environment and force juvenile fish to shorten their estuarine residence and venture to sea before they are biologically ready (Pearcy 1992).

Plume

Because of the magnitude of the Columbia River outflow, relatively little mixing occurs in the estuary, and the flow is still made up primarily of freshwater as it enters the ocean. Physical and chemical boundaries characterize this discharge plume and may have a dramatic influence on the conditions

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controlling salmon survival during early stages of their marine life (Pearcy 1992).

Prior to the development of upstream storage reservoirs in the Columbia River, the coastal plume had two well-defined seasonal directions. In the winter, the plume extended in a northerly direction. In the summer, the direction of the plume reversed and the net transport moved toward the southwest (Ebbesmeyer and Tangborn 1992), protruding 300–400 km into the ocean. Dams and the storage of water reduced the peak flow of the Columbia River and disrupted this natural seasonality of the river's plume, particularly during the spring and early summer, when many juvenile salmon enter the ocean. Regulated flows also changed the salinity and density gradients of coastal waters off Oregon and Washington north and south of the Columbia River estuary (Ebbesmeyer and Tangborn 1992). Although the biological effects of these changes have not been studied, Pearcy (1992) proposed that the turbid discharge plume provides a mechanism to carry migrating smolts offshore, isolating them from visual predators while keeping them in highly productive waters.

Northeastern Pacific Ocean

The ocean environment utilized by Pacific salmon from the Columbia River Basin may be quite extensive and diverse because different populations follow distinct patterns of migration during their marine residence (Pearcy 1992). While some stocks remain nearshore, others range from northern California to the Gulf of Alaska. This broad marine area encompasses several ecological units, each characterized by distinct hydrographic regimes, bottom topography, productivity, and trophically dependent populations (Francis 1993). Variations in ocean conditions occur over relatively short periods of a few years, as well as over longer term cycles measured in decades. Within any time period, geographic variation in conditions can be pronounced as well.

Intense upwelling processes are perhaps the most important oceanographic features controlling fish distribution, abundance, and feeding centers. Good upwelling conditions usually mean good survival of salmon (Pearcy 1992), although the mechanics for this relationship are still speculative (Brodeur and Pearcy 1992). The expansion of hatchery releases in the Columbia River Basin in the 1960s and 1970s, for instance, raised concerns that the competition between salmon of different origins could result in food shortages, particularly in years of poor upwelling. Other studies suggest that the effect of upwelling on salmon survival is linked to the functional response of predators to salmon and alternative prey organisms. Years of high upwelling may disperse salmon and provide alternative prey, while years of weak upwelling result in higher predation on juvenile salmon (Pearcy 1992). Evidence for and against these arguments is still inconclusive. A clear assessment of these interactions may help define the spatial and temporal variability of the ocean environment.

Climate change is another source of variability in the functioning of marine ecosystems, in general, and salmon production, in particular (Beamish and Bouillon 1993). One of the most drastic short-term perturbations of the northeastern Pacific is the El Niño condition, a recurring phenomenon with an irregular interannual periodicity of 2–7 years (Mysak 1986). El Niño events may sometimes lead to local reversals in tropical Pacific wind and ocean currents which may alter surface water temperatures and the distribution of upwelling and productivity along the Pacific Coast. Changes in species composition and abundance can also be pronounced during El Niño events (Brodeur and Pearcy 1992).

A large-scale feature responsible for much of the climatic variability observed in the North Pacific is the atmospheric low pressure system centered over the Aleutian Islands (Beamish and Bouillon 1993). This system alternates between two generalized locations and affects the split between the Alaska and California currents on annual to decadal time scales. The location of this oceanographic split may be critical in determining the seasonal and interannual biological characteristics off Washington and Oregon (Gargett 1997).

Recent insights into the configuration of the Aleutian low pressure system have provided evidence for the analysis of regime shifts in the ocean climate between the north and south ends of the North Pacific (Mantua et al. 1997). The Aleutian low pressure system dominating most of the North Pacific from 1976 to 1977 is of singular importance because it was the most intense low since 1940–1941 and signaled a change in the pattern of the North Pacific climate (Beamish 1993; Hare and Francis 1995). Many West Coast salmon populations appeared to respond to this shift. Sharp decreases in abundance have been observed in many southern salmon populations (south of Vancouver Island, British Columbia) and dramatic increases in northern populations (north of Vancouver Island, British Columbia). The recent period (post-1976) has been characterized by successive years with a deep wintertime Aleutian Low. This change in the atmospheric circulation has resulted in a prolonged period of warmer upper ocean temperatures in the northeastern Pacific (Hare and Francis 1995; Mantua et al. 1997). Gargett (1997) proposed that the interdecadal alternation of the Aleutian Low affects the stability of the water column and plankton production, and this may further explain the inverse relationship between the abundance of salmon in the Gulf of Alaska and those from the Oregon and Washington coasts.

Interaction between salmon and environmental variability

Variation within the marine environment is particularly important in determining the success of individuals, populations, and species of anadromous salmonids because they spend most of their life in the ocean. Salmonids can be quite plastic in their response to environmental change and accommodate this variability with a relatively high degree of genotypic and phenotypic diversity (Thompson 1991; Adkison 1995; Healey and Prince 1995). Within salmonids, features such as spawning, dispersal, morphology, maturation, and patterns of growth are all life history traits that define the "fit" of individuals to their environment. Each life history represents a suite of characteristics that defines the episodes of birth, reproduction, and death of individuals and the dynamics of populations over evolutionary time.

Variation in life histories represents different biological solutions to intrinsic environmental variability. In a natural

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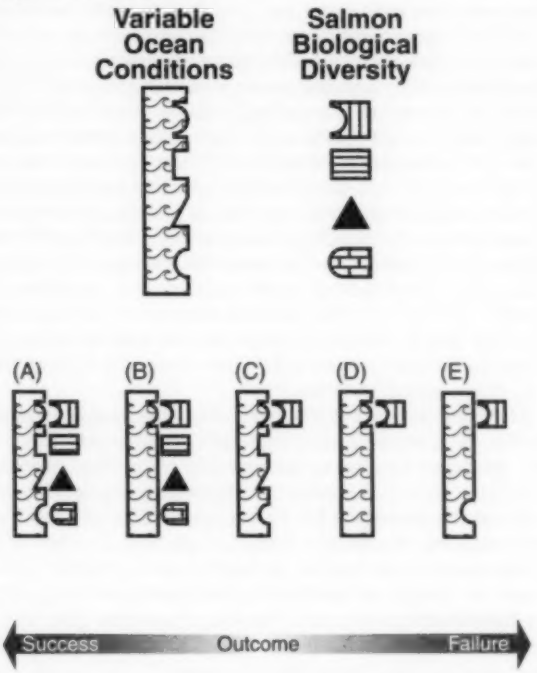
Fig. 1. Interaction between ocean conditions and salmon life history diversity. Five possible scenarios (A–E) illustrate the relative definition of failure and success of salmon management as determined by the fit between different salmon life histories (shapes on the top right) and the variability in ocean conditions (template on the top left).



ecosystem, life history strategies evolve through natural selection, operating under anatomical, behavioral, and genetic constraints, to match key characteristics of the environment. When confronted with variable environmental conditions, however, no one solution is always optimal. Hence, depending on the particular environmental template (sensu Southwood 1977) encountered by individuals, certain life histories are more successful than others and convey a reproductive advantage and increased fitness (Thompson 1991). The environmental template varies in time and space and determines the range of possible life histories needed to maintain fitness through time (Southwood 1977).

Human interventions add important hurdles to the ability of salmonids to maintain their biological diversity and, hence, their ability to withstand environmental variation. Anthropogenic forces may act as agents of artificial selection (Sheridan 1995), favoring some life history traits over others, and can modify the generalized anadromous life cycle of Pacific salmon. Fish husbandry practices, for example, can select against behaviors and morphologies that may be advantageous in the wild but that are less conducive to the operations and goals of hatchery programs (Reisenbichler 1997). Harvest, hydroelectric dam operations, flow manipulations, and many other human-driven perturbations can act synergistically with variable environmental conditions to alter the biological structure of salmonid populations. Under favorable environmental conditions, salmon populations show a higher tolerance to human perturbations. When environmental factors are restrictive, the relative importance of human factors increases and the capacity of salmon populations to tolerate external disturbances becomes very limited (Fogarty et al. 1991).

Intensively regulated freshwater systems tend, in general, to reduce environmental variation (Stanford et al. 1996). Floods are reduced, banks are stabilized, and biological components are adjusted to fit within the needs of a heavily engineered river redesigned through human technology. For example, in the Columbia River, a variety of actions are taken to enhance survival of juvenile fish during their downstream migration. These include seasonal flow augmentation, controlled spill at hydroelectric dams, and physical transport of fish around dams in barges and trucks (Ebel et al. 1989). Many of these actions are designed to force a particular biological configuration that minimizes the conflict with other human uses of the river. Because these actions are expensive, their implementation tends to be optimized on the basis of juvenile fish abundance rather than on the natural diversity of salmon life histories. Based on the relationship between the habitat template and ecological strategies (Southwood 1977), simplification of the environment and its variability also should result in a decrease in biological diversity (Stanford et al. 1996).

The interaction between environmental variability and biological diversity is illustrated in Fig. 1. Environmental variability is depicted on the left as a template (Southwood 1977) with variously shaped indentations representing combinations of spatial and temporal environmental conditions encountered by salmon over the course of their life cycle. This variability promotes the development of adaptive mechanisms that maintain relative fitness in salmon populations and shapes the evolution of life history traits (Thompson 1991). Different life histories are represented by correspondingly shaped pieces on the right. As an adaptive mechanism influencing reproductive success and fitness, the correspondence between the life histories and the environmental template must ultimately embrace the entire life cycle. The environment at each life stage, however, is realized by salmon at different scales and offers its own unique set of factors and circumstances (Levin 1992). Thus, it is possible to imagine a different template for the environment encountered at each life stage. For purposes of this discussion, the templates in Fig. 1 illustrate the variability in the ocean environment encountered by populations of juvenile salmon entering from the freshwater (in this case the Columbia River). Five general scenarios are discussed below, focusing on variation in ocean conditions, life histories, and their significance to salmon survival.

Figure 1A represents an ideal scenario where favorable ocean conditions provide adequate survival and growth opportunities to a broad range of salmon life histories. Due to the complex and dynamic nature of the environmental template, however, even "favorable" conditions can be better described as some average state tracked by the bulk of available life histories. In this ideal case, variation in the shape of the template results in varying degrees of match and mismatch between some life histories and the environmental template. Different life histories may be favored according to random circumstances and decadal or longer environmen-

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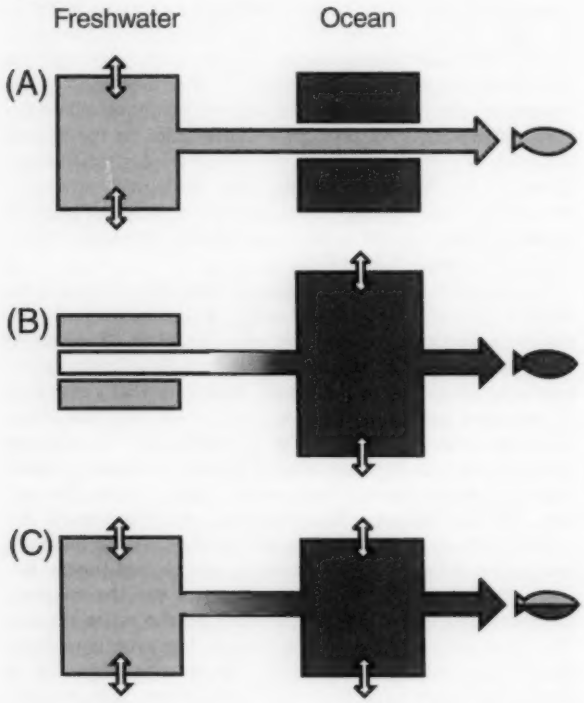
Fig. 2. Different theoretical perspectives on the relative impact of freshwater and ocean environments on salmon production: (A) freshwater dominance, (B) marine dominance, and (C) holistic. In each case, the box on the left represents the freshwater environment, while that on the right represents the marine environment. The horizontal arrows through the boxes illustrate movement of juvenile salmon during their life cycle from freshwater to the ocean. Vertical arrows on either the freshwater or marine boxes indicate where adjustments may occur in order to affect the number of fish returning. These adjustments can be natural, in the form of environmental variability, or human caused, resulting from management actions to increase numbers of fish or other activities that reduce their abundance.



tal cycles. Therefore, the evolution of a rich mosaic of life history strategies provides salmon with their characteristic resilience to a variable environment. In other words, "...a disturbance regime that can be incorporated is not disturbing at all" (Urban et al. 1987).

Figure 1B depicts an extreme condition where the environmental window of biological opportunity is severely narrowed, perhaps as a result of an El Niño episode or other climatic event. While some of the available life histories fit the template, many others are disfavored, resulting in decreased salmon abundance. In fact, the persistence of some life histories may be in jeopardy; however, as the environmental template varies again, these life histories may reappear to take advantage of new opportunities. Over time, a biologically diverse array of life histories exists to accommodate adverse environmental demands.

As discussed above, human interactions with the ecosystem tend to reduce biological diversity. This makes the natural mechanism for incorporating environmental variability less effective (Urban et al. 1987). This situation is depicted first in Fig. 1C where a reduced set of life histories fits segments of the environmental template. The outcome of this scenario is probably a reduced abundance relative to the situation in Fig. 1A or even Fig. 1B, although artificial augmentation can bolster abundance under favorable conditions. However, some might conclude that any reduction in fish abundance is simply a necessary sacrifice towards progress and point to the apparent success of mitigation efforts directed at the reduced set of populations. A strategy of this kind, however, may prove risky and result in serious ecological imbalances, as illustrated in the next two scenarios.

For example, it is possible that a much reduced set of life histories can thrive during a period of generally unfavorable ocean conditions if there happens to be a fortuitous fit between the available life histories and the ocean conditions (Fig. 1D). Then the end result parallels the outcomes described for Fig. 1C. The apparent success of a management strategy that focuses on customizing fish populations to the assumed "normal" ocean conditions masks the serious risks of environmental variation.

If the environment shifts away from the situation depicted in Fig. 1D, then the limited set of life histories may fail to fit the template of ocean conditions (Fig. 1E). This mismatch translates into a disastrous recruitment collapse. Because there are no alternative life histories to exploit the prevailing environment, abundance declines, perhaps precipitously. Some populations can be extirpated, and, without a rich source of biological alternatives, recolonization can be slow or nonexistent.

Taking ocean conditions into consideration

The question of how ocean conditions can be taken into account for salmon management revolves around how we view the relationship between marine and freshwater environments and their relative significance to salmon. Figure 2 illustrates three different possibilities for this relationship, each of which results in an alternative approach to salmon management. While no individual or entity necessarily ascribes fully to any of these hypothetical views, elements of one or more of them form the basis for many salmon management decisions in the Columbia River Basin and elsewhere. These explanations for, and proposed responses to, the fluctuations in salmon abundance have polarized numerous interest groups, administrative entities, and segments of the public involved in the architecture and implementation of salmon recovery programs.

Freshwater dominance

The first view (Fig. 2A), which characterizes traditional salmon management for most of this century, suggests that fluctuations and declines in salmon abundance are largely the result of deterioration of the freshwater environment due to development activities. While acknowledging that a large part of the mortality that occurs over the course of the salmon life cycle takes place in the ocean, the implied assumption is that the ocean is a relatively stable environment where salmon mortality affects a constant proportion of

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smolts entering the ocean. It then follows that fluctuations in the production of adult salmon can be dampened and declines can be reversed through the manipulation of the smolt output (Bottom 1997). From this "freshwater dominance" perspective, management actions in freshwater areas above the estuary have a direct impact on overall salmon production. According to this argument, a management course that relies heavily on artificial production and technological innovations, for instance, is generally correct. Hatcheries and hydroelectric operations are managed to provide a standard "product" with the focus of increasing the number of juvenile fish entering the ocean. Augmenting the number of juveniles released from hatcheries is the strategy of choice to increase numbers of fish available for harvest or returning to the river, independent of variation in ocean conditions (Bottom 1997). Ironically, continued declines in fish runs do not provide the much needed evaluation of the strategy, conceptual criticism, or rejection of program activities. Instead, they seem to provide the necessary justification for a more aggressive implementation of the current technological fixes (Meffe 1992; Stanford et al. 1996; Lichatowich 1997).

Marine dominance

The second view pervading the political environment of salmon management ascribes most of the variability in salmon abundance to conditions in the marine environment (Fig. 2B). In many ways, this is the opposite of the first view. This "marine dominance" perspective views the ocean as the ultimate controller of fish populations. In this scenario, environmental changes in the ocean control the number of fish, and the freshwater environment is reduced in importance.

Because many sources of mortality affecting salmon during their freshwater phase are under management control, compared with very few in the marine environment, both the efficacy of freshwater actions as well as the importance of salmon mortality during their seawater residence are reevaluated under this view. Failure to observe simple cause-and-effect relationships between augmented smolt abundance in freshwater and eventual returns of adult fish suggests that variation in the abundance of salmon runs can be attributed to factors outside human control. It then follows that freshwater actions may assist downstream migrants and returning adults, but are relatively less important in the face of large and variable ocean conditions. The significance behind this argument is that if changes in the ocean climate dominate changes in salmon biomass, then actions to improve conditions in the river or its tributaries are relatively futile, particularly in years when ocean conditions are unfavorable. This view could lead to the conclusion that recovery efforts and funding for them may be wasted because ocean conditions negate the effect of any improvements in the freshwater environment.

Holistic

The first two perspectives view the freshwater and marine environments as distinct and separable habitats. Fundamentally, they differ in regard to the relative importance placed on either area as determinants of salmon abundance. In recent years, the continued decline of salmon from the Columbia River has called into question the wisdom underlying both of these management arguments. More recent thinking about ecosystems and their importance to species of interest, such as salmon, as well as a greater understanding of the ocean, leads to a third conceptual alternative (NRC 1996; Williams et al. 1996). Under this view, freshwater and marine areas are integral components of a larger ecosystem within which salmon exist (Fig. 2C). Hence, the abundance of salmon reflects the overall condition of the entire ecosystem and variation in both the freshwater and marine environments. This view reflects a greater appreciation of the ecological context of fisheries management (Bottom 1997). Variation in the environment, including ocean conditions, is a natural feature of the ecosystem to which salmon have adapted through a diverse array of biological traits. The shift of management focus toward the entire salmon ecosystem recognizes that even though the ocean is variable, management actions, particularly those in freshwater systems, are critical in promoting the conservation of this diversity over time (Lawson 1993).

This "holistic" view of the ecosystem can be summarized in the following points. (*i*) The ocean is not a constant environment. Ocean conditions and carrying capacity vary and can be limiting. (*ii*) Freshwater and marine environments are not independent. There is evidence that variations in the two environments are linked via large-scale atmospheric processes and that both are integral parts of the salmonid ecosystem. (*iii*) The estuary is an important transition between these two portions of the ecosystem. Conditions in the estuary can be an important determinant of early ocean survival of salmon. (*iv*) Environmental variability is an inherent feature of the ecosystem of salmon. Salmon accommodate this variability through a similar variety in life history traits.

These new understandings have significant implications for the management of salmon. The environmental and functional fragmentation of the salmon ecosystem, expressed in the first two views, leads to management approaches that have proven to be too narrowly focused. When the entire ecosystem of salmon is recognized as the organizing principle behind a balanced management framework, the sources of environmental and biological variability and the spatial and temporal scales involved acquire new significance. This leads to a comprehensive management approach where different parts of the salmon ecosystem are integrated for implementing adaptive management strategies.

Proposed management response

Although the causes of salmon mortality in the marine environment are difficult to study, our understanding of how ocean conditions affect long- and short-term variation in salmon populations has increased over the last several years. In the northeastern Pacific, in general, and the more localized realm of the Columbia River estuary and discharge plume, both natural phenomena and human interventions have modified environmental conditions and their rate of change. Although salmon tolerate a wide range of environmental conditions and disturbances, many factors may be lethal or cause physiological stress. The integration of these factors into management policies is based on our perception of the whole salmon ecosystem, a wide area that spans marine and freshwater domains.

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Based on an understanding of the mechanism that salmon use to cope with environmental variation (Fig. 1) and a holistic view of salmonid ecosystem (Fig. 2C), fish and wildlife managers may employ two primary approaches to influencing salmon survival in the ocean. The first approach is through the improvement of estuarine and nearshore conditions. The Columbia River estuary and nearshore plume are important to salmon production, particularly because of their impact on survival of juvenile fish making the transition to the ocean environment. Like many estuaries, these areas have been, and continue to be, negatively affected by upstream flow regulation, construction of dams, and local habitat change. Hatchery operations may also result in ecological imbalances, competitive interactions, and competition for food and space by smolts during their estuarine and plume residence.

Based on these points, consideration of ocean conditions could include evaluation of flow regulation, river operations, and habitat management in regard to their impacts on the estuary and nearshore marine areas. For example, efforts in the Columbia River to restore the seasonal hydrograph through release of stored water during the spring have generally been conceived as a way to assist downstream migrating salmon and steelhead (e.g., NPPC 1994). A more holistic view of the salmonid ecosystem suggests a broader biological role for flow including estuarine habitat and food web development and establishment of a river plume that approximates the condition under which Columbia River salmon evolved. Flow regulation to affect estuarine and nearshore areas may require volumes and schedules different from those used for upriver biological purposes. Estuarine habitats lost to past efforts to stabilize riparian areas and land reclamation can be reclaimed. In several northwestern rivers, for example, estuarine habitat has been reestablished by breaching dikes to allow normal tidal flooding of estuarine areas (Simenstad and Thom 1996). The key role of these areas in the critical freshwater–marine transition suggests the need for examination of these possibilities in the Columbia River as well.

The second management approach available to resource administrators is to address environmental variability, whether freshwater or marine, through the preservation of life history diversity in salmon, a natural survival mechanism that evolved in response to changing conditions (Thompson 1991). Fluctuations in the ocean climate are an integral component of the overall environmental variability encountered by salmon. Salmon and steelhead in the Columbia River and elsewhere accommodate environmental variability through the development of a wide range of biological traits and behaviors that have been selected to permit survival within this ecosystem. However, management actions often restrict the natural expression of salmon life history diversity. Actions that target limited time periods (e.g., restricted flow augmentation, spill, transportation, and hatchery release schedules), select for particular physical characteristics of the fish (e.g., harvest and hatcheries), or reduce complexity of habitats (e.g., reduction of seasonal flows and channelization) can restrict biological diversity. For instance, the current operation of bypass systems at dams, smolt transportation, flow augmentation, and spill, generally occurs within a relatively short time period from about April 15 to July 31 in the Snake River and from May 1 to August 31 in the Columbia River (NPPC 1994). However, the juvenile fish migration extends appreciably before and after this period. Some bypass measures select for some physiological and morphological conditions over others (Muir et al. 1990). To the extent that these actions enhance passage conditions for the majority of the migrating population, they select against life histories that migrate outside this window of time or are not captured by the actions. The result is a focusing of the migration within a narrow time interval and a selection against the wide range of migration strategies that might occur naturally. Therefore, a major option for taking ocean conditions into account involves ensuring that restoration strategies are designed and evaluated in regard to their potential to restrict or enhance the natural expression of biological diversity in salmon populations.

The hypothetical scenarios described in Fig. 1 have significant implications for the management of salmon, specifically those that limit salmon diversity. It is possible to take advantage of favorable ocean conditions and dampen negative ones by addressing potential settings of the ocean environment in a proactive, rather than a reactive or even a resigned, manner. An appropriate management response to environmental variability in general, and ocean variability in particular, is to relax anthropogenic pressures that inhibit or restrict development of a natural range of biological diversity.

From this premise, a primary means for taking ocean conditions into consideration in salmon management in general, and for the implementation of the Council's program in particular, it to evaluate recovery actions and their impact on biological diversity. Hatchery practices, for example, should need to minimize selection that restricts or skews the distribution of biological diversity. The role of hatcheries within a functioning salmonid ecosystem needs to be developed to allow populations to express diversity levels that enhance long-term survival (White et al. 1995). In the Columbia River, the use of smolt transportation, flow augmentation, spill, and other juvenile migrational aids should be managed to provide benefits to the entire spectrum of migrants rather than the central majority of the run. Restoration efforts need to move from the paradigm of management for the average biological condition to management for the range of potential biological variation (Williams et al. 1996). Common to these scenarios is that biological diversity is not "fixed" or engineered in a mechanical sense but is allowed to increase to the appropriate level by relaxation of anthropogenic constraints. The appropriate level of biological diversity is not static, if indeed it is even quantifiable or predictable, but varies according to short- and long-term trends in the environment.

In the debate over innovative approaches to salmon management, it is questioned whether current capabilities to forecast changes in the marine environment are sufficient to adjust management decisions (Pulwarty and Redmond 1997). In general, resource managers are concerned with anticipating ocean and atmospheric conditions to manage their coastal waters and resources. The advent of modern remote sensors and electronic instrumentation has expanded significantly the ability to sample ocean conditions, fish distributions, and ecosystems over multiple scales of space and time. Based on this progress, it has been argued that the im-

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pacts of future atmospheric and oceanic conditions on salmon populations could be modeled and, to some degree, predicted (Simpson 1994). With sufficient predictive power, it might be argued that hatchery releases and other practices could be managed in real time to maximize the fit between biological traits and the environment (Fig. 1). However, the accuracy and precision of these predictions still require much improvement before management decisions can be implemented according to forecasts of ocean conditions (Walters and Collie 1988). Furthermore, a potential danger associated with this is continuation of the implicit belief that we can engineer biological systems to simultaneously meet the goals of multiple use, sustainability, and fish and wildlife as well. Predictive power aside, practical difficulties associated with managing production areas often located several hundred miles upriver from the ocean and the need to manage and protect the remaining wild populations and genetic resources argue against the hope that fish might be engineered in response to real-time environmental predictions. While new technologies can be useful tools for ecosystem managers, caution should be exercised to avoid a "predict-and-control" approach as a surrogate to implementing sound ecological principles (Stanley 1995).

The ideas presented in this perspective argue against the default notion that salmon management activities are futile in the face of variable ocean conditions. If attention is focused on that portion of the marine environment that includes the estuary and nearshore plume at the mouth of the Columbia River, then there are important ways to directly address ocean conditions through the restoration of estuarine habitats. Specifically, we call for a significant reassessment of management strategies that directly affect these environments. We further suggest that ocean conditions be taken into account by promoting the naturally evolved strategy that salmon use to accommodate environmental variability. We propose that policy directives and management actions be geared to estuarine habitat restoration and to promoting the conservation of salmon biological diversity. Improving the understanding of these actions and the ways they force biological changes in salmon life histories can enhance the management of Pacific salmon. Certainly, restoration of habitats and modification of actions and strategies to foster development of a natural expression of life history diversity within Columbia River salmon will likely conflict with other uses of the river and involve potentially costly trade-offs. Thus, the management questions confronting policy makers require an understanding of the impact of ocean and freshwater factors on salmon and a willingness to devise necessary adjustments to meet these challenges.

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ERRATA

Erratum: Temperature-dependent allometric models to estimate zooplankton production in temperate freshwater lakes

Jason D. Stockwell and Ora E. Johannsson

Ref: Can. J. Fish. Aquat. Sci. **54**: 2350–2360 (1997).

The subheading in the right-hand column of page 2352 should read "Model Development" and not "Modeloy Development."

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Erratum: Factors affecting the production of zooplankton in lakes

B.J. Shuter and K.K. Ing

Ref: Can. J. Fish. Aquat. Sci. **54**: 359–377 (1997).

The equation in the heading for Table 7 is incorrect. The heading should read:

Table 7. Comparison of three sets of estimates for the relationship: $\log([P/B]_{daily}) = \alpha_{taxon} + \beta \cdot T_{daily}$), where α_{taxon} is an additive constant specific to each taxonomic group.

Also, the left-hand side of eq. 15 is incorrect. The equation should read:

$$\log_{10}(gs) = \alpha_{taxon} - 0.020 \cdot T_{gs} + 0.133 \cdot \log_{10}(chl) + 0.047 \cdot \log_{10}(M_{ad}).$$

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